SPECIATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation entitled **"SPECIATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL"** is the bonafide original work done by **Dr.K. CHANDRASEKARAN,** Post graduate in Microbiology, under my overall supervision and guidance in the Department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation **"SPECIATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL"** is the bonafide work done by me at the Department of Microbiology, Government. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr.RADHIKA KATRAGADDA, M.D.,** Professor & H.O.D of Microbiology, **Dr. K.V. LEELA, M.D., DGO.** Professor, Department of Microbiology and **Dr. THYAGARAJAN RAVINDER, M.D.,** Professor of Microbiology Department of Microbiology, Govt. Kilpauk Medical College, Chennai - 600 010. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of Degree of M.D. Branch IV Microbiology examinations to be held in April 2017.

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INTRODUCTION

Enterococci are commensals of the gastrointestinal tracts of animals from simple invertebrates to humans[1].They are known to be relatively avirulent in healthy individuals, but have become important opportunistic pathogens, especially in hospitalized patients[2]. They are recognized as opportunistic pathogens and are natural inhabitants of the oral cavity, gastrointestinal tract and the female genital tract in both humans and animals[3]. They have emerged as important nosocomial pathogens[3,4].

The most frequent infections caused by these organisms include urinary tract infections, intra abdominal and intra pelvic abscesses [3,5,6]. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis, and rarely meningitis[5].

Although about 23 species of *Enterococci* have been identified, there are two main species, *Enterococcus faecalis* and *Enterococcus faecium* that are responsible for most human *enterococcal* infections[3,4,5]. Nevertheless, the incidence of other species of *Enterococci* is underestimated because of frequent misidentification [2].

Enterococcus was normally ignored by many clinicians in clinical samples as it is normal flora[7]. But now it stands individually in most of the recurrent infections and interferes with the healing of wound [7]. This organism has been reported as the second leading cause of nosocomial urinary tract infections and third leading cause of nosocomial bacteraemia in hospitalized patients[5]. This bacteria which is so far considered as a commensal has reached the status of highly virulent

pathogen [7].The natural ability of *Enterococci* to readily acquire, accumulate, and share extra chromosomal elements encoding virulence traits or antibiotic resistance genes lends advantages to their survival under unusual environmental stresses and in part explains their increasing importance as a nosocomial pathogens[6]. Longer hospital stay and immunocompromised conditions are known risk factors for nosocomial infections like *Enterococcal* infections[6].

Enterococci also show intrinsic ressistance to a number of commonly used antibiotics particularly the cephalosporins[6]. *Enterococci* are intrinsically resistant to many antimicrobial agents and this intrinsic resistance to commonly used antimicrobial agents may have allowed them a cumulative advantage for further acquisition of genes encoding high level resistance to aminoglycosides, Pencillins, tetracycline, chloramphenical,and now Vancomycin[6]. Moreover, the acquisition of high level aminoglycoside resistance and vancomycin resistance has limited the therapeutic options available and infections have become extremely difficult to manage[6]. Multidrug resistant *Enterococci* are emerging as a leading nosocomial pathogen[8].

Vancomycin resistant *Enterococci* (VRE) have emerged as important nosocomial pathogens in the last two decades throughout the world[9.] Since first reported in 1988, VRE has rapidly become one of the leading causes of nosocomial infection and major growing problems in health care facilities globally[10]. Emerging

Vancomycin resistance among *Enterocci* is a cause of concern as this leads to a great difficulty in treating the serious infections caused by them [9].

Vancomycin resistance is undoubtedly the greatest concern, associated with severe underlying disease, compromised host defences, indwelling urinary or central venous catheters (CVC), prolonged hospitalisation and administration of multiple antibiotics especially vancomycin and cephalosporins[11]. *E. faecium* is more resistant species than *E. faecalis* and emergence of vancomycin resistance in it has caused an increase in frequency of its isolations[2]. Nevertheless, the incidence of other species of *Enterococci* is underestimated because of frequent misidentification [2]. Hence proper identification to species level is essential for proper management and prevention of this bacterial infection in any health care institution[2]

The prevention, control and spread of multidrug resistant enterococci require coordinated effort from various departments and this can only be achieved by educating hospital staff regarding the problem of drug resistance, vigilant use of antimicrobials, early detection and reporting by laboratories and immediate implementation of appropriate infection control measures.

The above details clearly emphasize a need for isolation and identification of *Enterococci* to species level from various clinical samples so as to determine the

epidemiological pattern in our hospital. Hence it is an essential part of the surveillance system of each and every hospital setup to monitor continuously such VRE infections and to assess the antibiotic susceptibility pattern of VRE isolates. Assessment of the prevalence and changing trends of VRE infections are of immense help in planning infection control measures which should be implemented in the hospital and also in the community to reduce the mortality and morbidity caused by these VRE infections.In view of the above perspective, the present study is carried out in our tertiary care hospital in Chennai, India.

AIM & OBJECTIVES

1. To isolate and speciate the *Enterococci* from various clinical samples.

2. To detect the virulence factors of isolated *Enterococci* Species.

3. To find out the antibiotic susceptibility pattern and their emerging resistance.

REVIEW OF LITERATURE

Enterococci are common inhabitants of human and animal intestinal flora. Infections with Enterococcus species including *E.faecalis* and *E.faecium* have attained greater clinical importance particularly with the emergence of resistance to Vancomycin and they are nowadays mostly associated with nosocomial infections[12,13].

HISTORICAL PERSPECTIVES:

- In 1899, Thiercelin used the name *Entercocccque* for the first time in an article published from France. The name *Entercoccci* was proposed to gram positive diplococci to highlight its intestinal origin.[14,15] In 1906 Andrews and Horder coined the name *Streptococci faecalis* which caused endocarditis fermented lactose and mannitol but not raffinose. The role of Enterococci in urinary tract infection was also reported.[14,15] In 1919, Orla &Jensen used *Streptococci faecium* a different teriminology, which differed from *Streptococcus faecalis* by its fermentation pattern[14,15].
- In 1937 Sherman separated *Streptococci* into 4 groups as pyogenic, viridans, lactic and enterococcus. Latter term *Enterococcus*, characterstics of these organisims were considered as growth at 10^0C and 45^0C, which even survived for 30 minutes at 60^0C , shown growth in 6.5% Nacl (sodium chloride) and can grew at pH of 9.6 and its ability to split esculin[14]

- In 1937 Sherman & Wing proposed third species *Streptococcus durans*. In 1955, *Enterococci* from Gouda cheese was noted and was named *S.malodoratus* (meaning bad odour).[14,16] In 1959 some motile enterococci were noted producing yellow pigment and in 1968 those were named as *S.faeceum var casseliflavus*[17] In 1967, Nowlan and Debeil noted some strain of *Enterococci* were found to react with Group D as well as with Group Q , as they resembled closely with *Enterococci* from chickens they were termed as *Streptococcus avium*[14].
- In 1970, it was proposed by Kalina that a Genus *Enterococci* was to be established based on Phenotypic Characteristics & its cellular arrangement[15]. Farrow et al. in 1983 presented biochemical and DNA hybridization data indicated that *S.faecalis, S.faecium, S.casseliflavus, S.avium, S.durans*, and *S.faecalis var malodoratus* were all distinct.
- In 1984 Schleifer and Kilpper-Balz using DNA-rRNA and DNA-DNA hybridization observed that *S. faecalis* and *S.faecium* were very distantly related to other streptococci group including *S. bovis*, and suggested that it should be transferred to another genus , and it was this genus name "*Enterococcus"* was strongly proposed by Schleifer and Kilpper-Balz[18]
- Later Collins, Jones and Farrow with Schleifer and Kilpper-Balz worked it out with similar methodology and the newer names proposed were *E. avium, E. casseliflavus, E. durans, E. malodoratus,* and *E. gallinarum*. Though it was not incorporated into the official Bergey's nomenclature, but this work was cited in Bergey's Manual and considered to prop up the new genus

creation to include the *Enterococcal* group of organisms. Further Nucleic acid studies used to define other species within the proposed genus *Enterococcus*.[14]

HABITAT

Enterococci present widespread in nature and are found in soil, water, food, plants and animals including reptiles, insects, birds & Mammals. Like in other animals, In humans also they are predominainantly present in Gastrointestinal tract, less commonly inhabitate other sites like genitourinary tract and vaginal cavity. Many intrinsic characteristics of Enterococci make them to survive and grow in harsh environments[T]. They are adapted to those ecologically varying complex environments which may be enriched with nutrient or even depleted with oxygen.[19]

They are Gram positive cocci occurring in stool with concentration ranging from 10^5 to 10^7 CFU/gm[19]. *E. faecalis* is more common than rest of the species. *E.faecium* is found with an average count of 10^4 to 10^5 CFU/gm of stool [14]. *Enterococci* of animal origin may give an atypical reaction in diagnostically important carbohydrate reactions. The subsistence of these host related traits might provide valuable evidence in the investigation of the probable transfer from animal strains to humans[14]

TAXONOMY [20,21,22,23,24]**:**

Kingdom	:	*Bacteria (Cellular)*
Phylum	:	*Firmicutes*
Class	:	*Bacilli*
Order	:	*Lactobacillales*
Family	:	*Enterococcaceae*
Genus	:	*Enterococcus*

Members of the Genus Enterococcus[23]**:**

E. faecalis, E.faecium, E.avium, E.casseliflavus, E.cecorum, E.dispar, E.durans, E.gallinarum, E.hirae, E.munditi, E.malodoratus, E.pseudoavium*, E.raffinosus, E.saccharolyticus*, E.seriolicida, E.solitarius, E.columbae*, E.sulfureus*, E.asini and E.haemoperoxidus*.*

*Are not isolated from human beings.

Species are grouped on the basis of 16 S rRNA sequencing[25]

1. **Faecium group** : *E. faecium, E. munditi, E. durans, E. hirae*
2. **Avium group** : *E. gallinarum, E.casseliflavus*
3. **Cecorum group** : *E. cecorum, E. columbae*
4. **Other distinct** : *E.faecalis,E.dispar,E.sulfureus, E.saccharolyticus*

GENUS DESCRIPTION:

Enterococci are gram-positive cocci, oval in shape, arranged as pairs or short chains[26]. They are facultative anaerobes, non motile, catalase negative, react with Lancefield group D antigen, grow at 10ºC and 45ºC. Grow in broth containing 6.5% NaCl, at pH 9.6 and survive heating at 60ºC for 30 minutes, hydrolyse esculin in the presence of 40% bile, hydrolyse L-pyrrolidonyl -naphthylamide (PYR) to produce pyrolidonyl arylamidase[26].

Enterococci differ from other Group D faecal *Streptococci* like *S.equinus, S.bovis* by surviving heat for 30 min at 60ºC, growing in 6.5%NaCl broth, forming NH_3 from arginine[26].

VIRULENCE FACTORS:

Three main groups of virulence factors may enhance the capability of enterococci to colonize the gastrointestinal tract and cause disease[27]. The potential virulence factors (*Enterococcal* secreted factors, *Enterococcal* surface components) identified in *Enterococcal* isolates and proposed to play a role in pathogenesis are as follows [23,27,28]

Enterococcal secreted factors:

The first group, *Enterococcal* secreted factors, are molecules released outside the bacterial cell that contribute to the process of infection. A heterodimeric toxin Cytolysin /hemolysin, secreted by some strains of *E.faecalis* lyses human, bovine, rabbit and equine erythrocytes (but not RBCs of sheep) and polymorphonuclear leucocytes & macrophages, play major role in endocarditis and endophthalmitis rabbit models[23]

Enterococcal proteases-The serine protease (SprE) and gelatinase (GelE), of *E.faecalis* mediate virulence by several mechanisms like modification of immune components and degradation of host tissues [27]. This enzyme an extracellular zinc-endopeptidase, capable of hydrolyzing gelatin, casein, collagen, hemoglobin and other small biologically active peptides[19].

Extracellular superoxide, by most of *E.faecalis and some E.faecium* strains produced in huge amounts that may increase its virulence in mixed flora abcesses [23]. An extracellular metallo endopeptidase, Coccolysin secereted by *E.faealis* strains by inactivating the vasoactive peptide –endothelin mediate its virulence [23].

Enterococcal surface proteins:

Second groups, Enterococcal surface component, are thought to contribute to bacterial attachment to extracellular matrix molecules in the human host [27].

Aggregation substance –a surface bound protein encoded by Phreomone responsive plasmids which increases its adherence to epithelial cells and increases clumping of organisms and thereby facilitating plasmid exchange . In rabbit endocarditis model, it also promotes the growth of cardiac vegetations [19,23,].

The surface proteins – Acm (homologue adhesion of *E.faecium)* and Ace (adhesion of collagen of *E.faecalis*) are *Enterococcal* surface components detects adhesive matrix molecules (MSCRAMM) which mediate attachment of bacteria to host proteins like fibronectin, fibrinogen, collagen[27].

Other surface proteins sharing similar homology to MSCRAMM playing role in bacterial attachment and in virulence are second collagen adhesion of *E.faecium* (Scm), surface proteins (Fms) of *E.faecium*, SgrA (which binds to basal

lamina components), *Enterococcal* surface protein (Espfc) of *E.faecalis* and of *E.faecium*(Espfm) and EcbA which binds to collagen type V [27, 28]

Lipoteichoic acid (group D antigen) acts by modulating immune response by inducing the Interferon production as well as TNF thus plays its role in virulence [23]. Pili are important mediator for bacterial attachment and invasion into the host tissue. It is present in both *E.faecalis* and *E.faecium* and are thought to be targets of immunotherapy[27].

Biofilm Formation:

Biofilm producing bacteria are capable of adherence to damaged tissue and plastic surfaces such as medical devices. It helps them for colonization as well as increases their capacity to cause infections. Some strains (Mutants) of *E. faecalis* which lack pili have shown attenuated production of biofilm in experimental urinary tract infections and endocarditis[27]. Polysaccharides may interfere with phagocytosis and thus play its role in pathogenicity. *E.faecalis* some strains possesses 3 distinct classes of capsular polysaccharides which are considered to be the targets for immunotherapy[19,27]

Other virulence factors:

The third group of virulence factors has not been well characterized[27]. *E.faecalis* stress protein Gls24 – play a role in *Enterococcal* resistance to bile salts hence significant in pathogenicity of endocarditis[27]. *hyl Efm*- containing plamids found in *E.faecium* , increase the colonizing capacity[27]. Recently, a gene (AsrR) which encodes for oxidative stress regulation has been detected as an significant virulence factor of *E. faecium*.

PATHOGENESIS:

Enterococci are opportunistic nosocomial pathogens, infections may arise from translocation of its cells from the site of colonization of the gastrointestinal system to other host sites and even to the hospital setting [29]. From the site of colonization, the microorganism must evade host clearance and produce pathologic changes in the host, either by direct toxic activity or indirectly by inducing an inflammatory response[28].

Adherence to the host tissue is the first step for *Enterococci* to act as pathogens. During tissue invasion organism faces an environment unlike that of its site of colonization by producing higher level of redox potentials, reduced essential nutrients, phagocytic attack by leucocytes & other host defense mechanisms. *Enterococci* express factors that permit adherence to host cells and extracellular matrix, facilitating tissue invasion, effecting modulation in immune responses, and cause toxin mediated damage[19].

New traits Acquisition like virulence associated genes and their intrinsic resistance to the antibiotics that are used commonly, allow them to rise above the defense mechanisms of host. These acquired traits along with the acquired antibiotic resistance differentiate from the commensals and the virulent pathogenic strains. These along with the changed dynamics of host – commensal relationships like diminished host immunity, host injury and broad spectrum antibiotic use, promote colonization of new niches favoring infections and its dissemination to other tissues and environment[28]**.** Especially the use of drugs like cephalosporins (broad spectrum antibiotics) which are excreted in bile also active against anaerobes

and gram negative bacteria, which eradicate the challenging mechanism of intestinal flora and also significantly increases the suppression of important immunological signals like lectin RegIIIγ, which keep *Enterococi* in low numbers usually[27]. The *Enterococci* resistance traits exhibit survival advantage for a long time in the host or persistent existence in such environment and play a significant role as prominent nosocomial pathogens[28]. Their clinical significane further increased by an important factor that *Enterococci* can transfer the resistant determinants to other Gram positive bacteria [29].

Because of bacterial genome sequencing, it is better understood about bacterial diversity, virulence, pathogenesis, evolution, and mechanisms of antibiotic resistance. For around 560 *Enterococcal* strains the genomic sequences are available at present. It gives new clues regarding the *Enterococcal* evolution from commensal to significant nosocomial pathogens [27].

CLINICAL INFECTION

Enterococci are known to cause a wide range of infections, practically infecting almost all human systems.

Urinary tract infections:

Urinary tract infection (NosocomialUTI) is the commonest infection caused by the *Enterococci* . *They are* usually related with anatomic abnormalities of genitourinary tract, indwelling catheters, other instrumentation, recurrent UTIs and usage of prior antibiotics [27]. *E.faecium* being the predominant species (40%) followed by *E.faecalis* (25%) and other species (35%) being isolated[29]. In differentiating true infection from colonization may be really difficult and the

factors like presence of leucocytes in urine along with systemic manifestations like fever, other local symptoms and signs , and a colony count of $> 10^5$ CFU/ml may assist in this regard[27,29]. Removal of catheter itself may be just enough to eradicate this agent. The complications associated with *Enterococcal* UTI are recurrent bacteremias, pyelonephritis and perinephric abcess [27].

Bacteremia and Endocarditis:

Bacteremia without endocarditis is one of the commonest presentations of with *Enterococci*. Intravascular catheters and other devices are the most common sources and others like genitourinary and biliary tracts, pelvic and intra abdominal foci, UTIs, wound and bone infections also may contribute to bacteremic episodes. Bloodstream infections with *E.faecium* may carry the worse prognosis because of its higher rate of ampicillin and vancomycin resistance leaving fewer therapeutic options available[27]. The association of *Strongyloides* hyperinfection syndrome in immune compromised individuals is well known. They are important causative agents of both community acquired and health care associated endocarditis, ranking next to *Staphylococci*. Malignant and inflammatory conditions of gut and procedures involving genitourinary or gastrointestinal tracts act as the source of origin. Usually in Male, elderly, debilitated patients with other comorbid conditions tends to be affected. It can affect both native as well as prosthetic valves. Commonly affected valves are mitral and aortic valves. *E.faecalis* is isolated more frequently in Community associated Endocarditis. Typically presented as a sub acute course with fever, malaise, weight loss, cardiac murmur and typical stigmata of endocarditis (e.g.petechiae, Roth's spots , Osler's nodes). Common complications

involved are Heart failure, embolic phenomenon. The most common end organ involved being brain in embolic manifestations[27].

Intraabdominal, Pelvic and Soft tissue infections:

Enterococci being a part of gastrointestinal tract commensal can produce spontaneous peritonitis in patients with cirrhosis and patients undergoing CAPD (Chronic ambulatory peritoneal dialysis). It is usually isolated along with enteric gram negatives and anaerobes from clinical samples from pelvic and intra abdominal collections[27]. *Enterococci* are commonly isolated from soft tissue infections, particularly those involving surgical wounds. *Enterococci* colonizes decubitus ulcer and diabetic foot and can be a source of bone infections[27]. *E.faecalis* is the common species isolated.

Meningitis:

Only 4% of all meningitis cases *caused by Enterococci*. The most common species isolated is *E.faecalis* followed by *E.faecium*. Spontaneous meningitis is a community acquired in patients with many comorbid conditions like renal failure, Diabetes, immune suppression , malignancy and in children with CNS pathology. In hospital acquired in the presence of shunt devices it causes Post operative meningitis. In both forms clinical features are similar acute course with fever, altered mental status and signs of meningial irritation. Complications include hydrocephalus, stroke and brain abcess [27,29].

Other infections:

It also causes neonatal infections like late onset sepsis, bacteremia, pneumonia and UTI in the presence risk factors like prematurity, low birth weight and indwelling devices. It also causes bone and joint infections.

Health Care associated Infections:

Health care associated infection also referred to as “nosocomial Infection” or “hospital acquired infection”, is an infection occurring in apatient in process of care in the health care facility which was not there at the time of admission.The ability of *Enterococci* to survive and disseminate in the hospital setting and their potential to acquire antibiotic resistance determinants makes their treatment in critically ill patients, really a difficult challenge[27]. The emergence and spread of VRE and multi-drug resistant *E.faecium* isolates increase in rates of nosocomial infections and worsens the situation further. Hence it accounts for more post operative complications and causing more treatment failure which increases the mortality. *Enterococci* rank third as the causative agents of hospital acquired surgical-site infections. National health Safety Network, USA indicated that *Enterococci* were the second most common cause of health care associated Infections after *Staphylococus aureus*[30]. Prompt detection of patients colonized with VRE along with effective and strict infection control measures can reduce the transmission of VRE and help in prevention of health care associated infections[31].

LABORATORY DIAGNOSIS:

Enterococci can cause serious infections including bacteremia, Endocarditis and urinary tract and wound infections. Treatment of infections caused by vancomycin resistant *Enterococci* (VRE) poses great challenge due to its multi drug resistance. Hence accurate and prompt identification of *Enterococci* (including VRE & MDR strains) are essential as it will aid in providing better patient care[32].

Collection, transport and storage of specimens:

The specimens commonly processed are urine, blood, wound exudates and secretions from other sites or swab specimens. The standard method of collection of these samples is adequate[28]. *Enterococci* are nonfastidious, quite resistant to unfavorable environmental conditions, no special methods of transport and storage of clinical samples are needed[28]. Specimens can be transported to the laboratory using any of the transport medium or on swabs that are kept dry. *Enrerococci* grown on agar slants of Brain heart infusion agar/Tryptic soy agar can be stored for several months at 4ºC[28]. They can also be stored in cryo preservative media at -20ºC for many years. The preferable methods would be storage as frozen cultures at - 70ºC in 10% skimmed milk solution containing 10% glycerol or in heavy cell suspensions made in defibrinated rabbit or sheep blood. *Enterococcal* cultures on lyophilization can be indefinitely stored[28].

Direct examination:

However, only a presumptive report of "presence of Gram positive cocci" can be given in case of non sterile specimens. With sterile clinical specimens the direct microscopic examination of gram stained smears will be helpful in

diagnosing *Enterococcal* infections[28] It becomes difficult to differentiate colonization and infection especially in urinary tract infection, the presence of pus cells will be the key, where direct examination have significant role[27]. In surveillance programs for detection of VRE, direct detection of *Enterococci* especially VRE from clinical specimens (feces, rectal swab) can be evaluated by using conventional and real-time PCR. For rapid detection and identification of major pathogens of nosocomial bacteremia using multiplex real-time PCR assay (Light cycler vanA & vanB assay)in whole blood is available nowadays [30]. DNA probes which are commercially available also useful for direct identification of *Enterococci* from Blood cultures[28].

Cultural Characterstics

Enterococci are facultative anaerobic organisms and are less fastidious than other Streptococci (not requiring CO2 enriched environment). They grow readily on ordinary nutrient media, MacConkey's media and blood agar at a temperature 35°C to 37 °C[23].

On MacConkey agar, they form small (0.5 to 1 mm) magenta coloured colonies, due to lactose fermentation[26]. On blood agar, they form small (1-2 mm), circular, translucent and convex colonies with regular margins, usually colonies are non-haemolytic, but alpha and beta haemolysis are also observed[26]. One-third of the *Enterococci* species can produce haemolysis on blood agar. *E.durans* are β-haemolytic. *E. faecium* is often α-haemolytic. *E. faecalis* is usually non-haemolytic but may be β-haemolytic, which can be used as a differentiating feature. All other

species are α- haemolytic or non-haemolytic[23]. α-haemolysis is not due to the cytolysin productionbut due to the production of peroxidases. This haemolysis is due to the production of haemolysin, which is transmitted through a plasmid.

Selective Media

Selective media usually consists of a selective agent, such as sodium-azide, an antibiotic (usually kanamycin or gentamicin) or bile salts or 6.5% Nacl and an indicator such as esculin or tetrazolium, incubation at an elevated temperature,also has a selective effect (42°C-45°C) Most often used selective media are Bile-aesculin azide agar[23,28,33], KF *Enterococcus* Agar[33,34], M-*Enterococcus* Agar[33], KAAA (Kanamycin-aesculin azide agar)[35], Cephalexin-azetreonam-arabinose agar[23]

GENUS IDENTIFICATION - BIOCHEMICAL REACTIONS

Biochemical and physiological tests accomplish identification of *Enterococcal* species.

Presumptive tests for identification of Genus *Enterococcus* are Catalase test

Like *Streptococci*, *Enterococci* are catalase negative, although some strains do produce pseudocatalase [16,18,36]

Bile Esculin hydrolysis test[14,37] :

Enterococci were found to be bile tolerant (40% bile) by Weissenback in 1918. Esculin is a glycosidic coumarin derivative (6β-glucoside-7-hydroxy coumarin). Two moieties of the molecule (Glucose and 7-hydroxy coumarin) are

linked together by an ester bond through oxygen. For this test esculin is incorporated into the medium containing 40% bile. Esculin hydrolysis in the medium results in the glucose and aesculitin formation. This esculitin in turn reacts with the ferric ions (ferric citrate) to form diffusible black complex. It is considered positive, in the presence of 40% bile if esculin is hydrolysed to esculitin resulting in the blackening of the medium.

Positive control - ATCC strain of *Enterococcus faecalis*

Negative control - *Streptococcus agalactiae*

Growth In 6.5% Nacl broth [14,37]

In 1978, modified conventional medium of Facklam used by Quadri et al., They used 1% dextrose (instead of 0.1% dextrose) with bromocresol purple as pH indicator and the results were obtained in 8 to 24 hours. This test differentiates group D non-*Enterococcal* organisms *from Enterococci* species, e.g.*S.bovis*. Brain Heart infusion Broth with 6.5% Nacl with a pH indicator is used in the test. The positive test is denoted by turbidity and colour change of the indicator.

Positive control - ATCC strain of *Enterococcus faecalis*

Negative control - *Streptococcus agalactiae*

Heat Tolerance Test :

J.M. Sherman and co-workers demonstrated this property of heat tolerance in 1937. The methodology of the heat tolerance test has not been standardized. Some

laboratories heat a sample of broth culture to 60°C for 30 minutes and then streak the culture on blood agar plate.[14,26,38]

PYR Test :

R.R.Facklam in 1982, intially described this chromogenic enzyme substrate test. PYR reagent **(**α-pyrrolidonyl β-naphthylamide**)** impregnated in a filter paper disc and colour developer (p-dimethylaminocinnamaldehyde) is added separately. Results read within 4-5 min[39,40]. This test provides a presumptive identification of group A *Streptococci* and *Enterococci*.[39]

In this test, α pyrroglutamylaminopeptidase enzyme when present , PYR-substrate hydrolyzed to β- naphthylamide which is then identified by p.dimethylaminocinnamaldehyde (colour developer) and forms bright red colour product (red shiff base)[23,40]. This test can be done with PYR broth (Todd Hewitt Broth+ 0.01% α – pyrrolidonyl β - naphthylamide) and PYR reagent (0.01% p-dimethylaminocinnamaldehyde) The formation of cherry red colour after 4 hours of incubation in PYR broth taken as positive and formation of orange or yellow colour taken as negative reaction

Positive control : ATCC strain of *Enterococcus faecalis*

Negative control : *Streptococcus agalactiae*

Litmus Milk Decolarisation test

This is inexpensive and rapid test done first by Scherl and Blazevic. The test organisim was taken in a tube with litmus milk and incubated for 4 hours at 35-

37^{o}C, production of white or pale yellow colour is considered as positive and pink colour or No change in colour is taken as negative[17,41] .

Positive control : ATCC strain of *Enterococcus faecalis*

Negative control : *Streptococcus viridians*

SPECIATION OF *ENTEROCOCCI*

Facklam and Collins *proposed the classification of Enterococal* species can be into 5 physiological groups of species, based mainly on acid production from mannitol and sorbose and arginine hydrolysis[38]. Further speciation is based on acid production from 1% sugars like arabinose, raffinose, sorbitol, pyruvate, sucrose, trehalose and reduction of 0.04% tellurite, motility and pigment production

- **Group I** – It consists of nine species. They produce acid from sorbose and mannitol, arginine is not hydrolysed. *E.raffinosus* and *E.avium* are the clinically significant species in this group. Others are *E.pallens, E.gilvus, E.malodoratus, E.saccharolyticus, E.divriesei, E.pseudoavium* and *E.hawaiiensis.*
- **Group II** – It consists of eight species. They produce acid from mannitol only but not from sorbose and arginine hydrolysed. Most of the isolates recovered from human sources like *E.faecalis, E.faecium, E.casseliflavus, E.gallinarum,* belong to this group. Others are *E.mundtii, E.sanguinicola, E.haemoperoxidus, and E.thailandicus.*
- **Group III** – It consists of six species. They don't produce acid from sorbose and mannitol, but arginine hydrolysed. It includes *E.dispar, E.durans, E.hirae, E.ratti, E.canintestini,* and *E.villorum.*

- **Group IV** – It includes eight species. They don't produce acid from both sorbose and mannitol and arginine also not hydrolysed. It includes *E.cecorum, E.caccae, E.phoeniculicola, E.aquimarinus, E.asini, E.sulfureus, E.silesiacus and E.termitis.*
- **Group V** – It consists of six species. They ferment only mannitol producing acid but not sorbose and arginine not hydrolysed. *E.canis, E.columbae, E.camelliae, E.hermanniensis, E.moraviensis* and *E.italicus* are included in this group.

CARBOHYDRATE/SUGAR FERMENTATION TESTS

Mannitol fermentation was considered one important criteria in classification of *Enterococci* in early 1900s.[14] In 1920's Diebel, Ayers and Johanson did battery of test with sugars like arabinose, raffinose, mannitol, sorbitol, sucrose, lactose, maltose, trehalose, glycerol, inulin, and salicin along with other tests to speciate *Enterococci*. But their classification did not included the species faecium, equines and bovis.

R.R. Facklam in 1972 did a battery of 26 physiological tests for *Enterococcal* speciation. In this classification, faecium, casseliflavus, avium, or equinus species were detected[42]. Pyruvate fermentation, sorbose fermentation and arginine dihydrolation were added to the series by Gross in 1975. Following which in 1989, M.D. Collins and R.R.Facklam added the above three tests, along with a battery of 14 tests (from the classification of R.R. Facklam in 1972) to give a modified classification scheme of speciation by using conventional tests.[37] Kathryn L. Rouff et al., later in 1990, with the help of Facklam modified classification

scheme they identified most of all the currently described species[43.] *Enterococci* never produce gas as it lacks Kreb's cycle and respiratory chain. Fermentation test is done in brain heart infusion broth with 1% carbohydrates.Test organisms are inoculated and incubated for 24 hours at 35-37^{o}C and examined upto a week. Indicators used are bromothymol blue, bromocresol purple or Andrade's indicator. [23, 26]

Arginine Test[23,26]

In 1975, Kathryn Gross, observed that arginine hydrolysis can be used as one of the reliable test for *Enterococci* speciation[43]. In Arginine hydrolysis, arginine converted to citrulline is a dihydrolase reaction, where amino group is removed from arginine. Citrulline is then converted to ornithine, which undergoes decarboxylation and form putresciene.As decarboxylation occurs under anaerobic conditions tubes should be over layered with (1cm) liquid paraffin or sterile mineral oil.The medium turns yellow in initial stages of incubation because of small amount of dextrose getting fermented.Later due to the decarboxylation once the amines are formed, it returns to original purple colour.Moellers decarboxylase media is preffered media for this test.

Positive control : *Enterobacter cloacae*

Negative control : *Klebsiella pneumoniae*

Pyruvate Fermentation:

This is another important test used for *Enterococci* speciation. to differentiate *E.faecalis* and *E.faecium*. Overnight culture of test organisim was

inoculated onto Pyruvate broth (bromocresol purple as indicator) and incubated for 24 hrs at 35-37^{o}C upto 7 days[23]

Positive control	:	ATCC strain of *Enterococcus faecalis*
Negative control	:	ATCC strain of *Enterococcus* faecium

Motility - is method for identifying the motile *Enterococci* like *E.casseliflavus* and *E.gallinarum* determined by hanging drop, modified motility semisolid media, and wet mount preparation[44]

Pigment production – *E.casseliflavus* and *E.munditi* produces yellow pigment and it can be detected by growing the organisim in tryptic soya agar and it is observed using a cotton swab by touching up the growth from the culture plate.[44]

OTHER TESTS

Potassium tellurite Reduction

Diebel in 1964 observed reduction of 0.05% potassium tellurite as one of the physiological conventional tests to differentiate *Enterococci*. Facklam in 1971 and in 1989[18] employed 0.04% potassium tellurite in blood heart infusion agar (defibrinated blood) as one among the batteries of tests used to speciate *Enterococci*[43]. Conventional test method of Facklam and Collins was followed by Kathryn, L. Rouff et al., and they observed the ability of certain strains of *Enterococci* to grow and able to reduce potassium tellurite (0.05%) prepared in Todd Hewitt Broth. It was detected by blackening of the medium.

Voges-Proskauer Test

The production of acetyl methyl carbinol was tested for certain *Enterococcal* strains by Susan S. Fertally and R.R. Facklam in 1987 [39]. Cobelentz method is accepted method for *Enterococci*. After 24-48hrs incubation to glucose phosphate broth with test organism addition of 0.6ml of 5% alpha naphthol followed by 0.2ml of 40% potassium hydroxide in that order the appearance of red colour within 15 min considered as positive result.[36]

Some tests like growth of *Enterococci* at 10 and 45 ^{o}C , Sodium Hippurate hydrolysis, and 0.1% tetrazolium reduction are other tests satisfied by some *Enterococcal* strains[26]

COMMERCIAL IDENTIFICATION SYSTEMS-

Manual, semiautomated and automated systems like API 20S, API Rapid STREP, Rapid ID32 STREP, BBL Crystal gram positive ID system, Gram positive identification card of Vitek system, RAPID ID STR, MICRO SCAN Gram Positive Breakpoint Combo panel etc. are available. These are reliable for the detection of most common species *E.faecalis, E.faecium* and to a lesser extent other species of *Enterococci*[44].

TYPING METHODS [45,46]

Method	Principle	Discrimination / reproducibility	Applications
Ribo typing	Hybridization of labelled rDNA with digested genomic DNA	Medium / Good	Too low discrimination for outbreak analysis (short term epidemiology)
RAPID/ repPCR	PCR with random primers or primers binding to repetitive target sequences	medium / insufficient - good	Partly suitable for „in house“ outbreak analyses; provided with commercial kits (DiversiLabTM)
AFLP	Length polymorphisms in genomic PCR products	Good / good–very good	Exchanged by MLST due to better data portability and discriminatory power
PFGE	Genome-based macro restriction analysis	Excellent/ good–very good	Still “Gold-Standard” for outbreak analyses; not suitable for long term epidemiology analyses
MLST	DNA sequence comparisons of housekeeping genes	good – very good / excellent	„Gold Standard“ for population-based analyses; expensive and laborious, too less discriminatory for outbreak analyses
MLVA	Fragment length polymorphisms in genomic repeat regions	good – very good/ very good	Suitable for population-based analyses; too less discriminatory for outbreak analyses
vanA cluster typing	Different schemes exist based on amplification, digestion, sequencing3	good – very good / very good	Only suitable for specialist analyses and in combination with basic techniques (PFGE, MLST, MLVA)
Plasmid typing	Analysis of the plasmid content and composition	Limited / very good	Dependent on the corresponding question; suitable for analysis of “plasmid hospitalism” and for enhancing MLST/MLVA analysis’ results

Legend: AFLP, Amplified-Fragment Length Polymorphisms; MLST, Multi-locus Sequence Typing; MLVA, Multiple Locus Variable Number of Tandem Repeat Analysis; NGS, Next Generation Sequencing (synonymous for various techniques such as 454, illumina, ion torrent); PFGE, Genomic macrorestriction analysis in Pulsed-field Gel electrophoresis.

The pace with which MALDI –TOF MS that can scrutinize bacterial isolates for detection purposes and potentially for relatedness makes it as choice of alternative genotypic detection methods. It involves Low turnaround time, Less intensive labor[47].

EPIDEMIOLOGY

Enterococci are part of the normal intestinal flora in humans. The predominant species, *E. faecalis* and *E. faecium*, are the rising and leading causes of nosocomial infections[14,23].

UTls is the commonest *Enterococcal* infections among humans and they are commonly associated with the structural abnormality or instrumentation of the urinary tract. Intra abdominal and pelvic wound infections, bacteremia are the second and third most common infection of *Enterococci* respectively[23].

E. faecalis the most common species isolated from nosocomial infections, the isolation of *E. faecium* has rised considerably in the past two decades. In reality *E.faecium* at present is nearly equal to *E. faecalis* as an agent of etiology in hospital-associated infections. This fact is more significant, as *E. faecium* is considerably the challenging and most resistant species among *Enterococci* to treat[27].

Enterococci are important pathogens because of their resistance to multiple drugs. All *Enterococci* have intrinsic low-level resistance or relative resistance to penicillins, cephalosporins, aminoglycosides etc

In the past few years a remarkable increase in the Vancomycin resistant *Enterococci* has been reported. Between 1989 through 1993, nosocomial infection (due to VRE) has increased from 0.3% to 7.9% reported by National Nosocomial Infection Surveillance System, CDC. This was reported due to a rise in vancomycin resistant enterococci infections in intensive care units (ICU)[48].

Risk factors for enterococcal infections are long duration of hospital stay, patients with indwelling catheters, serious underlying diseases, immunosuppression (oncology and transplant patients), renal insufficiency, prolonged antibiotic therapy causing selective antibiotic pressure, particularly with broad-spectrum antibiotics like cephalosporins, imipenem, ciprofloxacin and aminoglycosides [48].

Both colonisation and infection of *Enterococci* expressing multi drug resistance occur worldwide. It is common in almost all patients those who are colonized or infected with vancomycin resistant *Enterococci* or multidrug resistant exposed to previous use of antibiotics. In Europe the use of glycopeptides (avoprocin) in animal feeds appear to be the key contributor to the emergence of the Vancomycin resistant *Enterococci*.[48]

In India, vancomycin resistance among the *Enterococci* is emerging. Various authors in their prior studies have reported prevalence ranges from 1-8.7% [49,50].

ANTIBIOTIC THERAPY

The most active among the β-lactams, are the aminopenicillins (amoxicillin, ampicillin) and ureidopenicillins (i.e., piperacillin); next active are penicillin G and imipenem[27]. For *E.faecium*, a combination of high-dose ampicillin plus an aminoglycoside has been suggested. For synergistic therapy only two aminoglycosides, Streptomycin and Gentamicin are recommended to treat severe *Enterococcal* infections. Tobramycin should never be used against *E. faecium*, use of amikacin is discouraged and monotherapy with aminoglycoside is not effective. An alternative to β-lactam drugs, Vancomycin is used for the treatment of *E.faecalis* infections but is also less useful against *E. faecium* as resistance becomes common[27]. Cephalosporins are inactive against *Enterococci* except ceftobiprole for *E.faecalis* infections. For the treatment of urinary tract infections single agent like nitrofurantoin, fosfomycin, ampicillin or amoxicillin can be used[27].

Linezolid and Quinupristin/Dalfopristin are FDA approved drugs used in some VRE infections, both are bacteriostatic drugs and used as an alternative to the standard drugs.

ANTIBIOTIC RESISTANCE

Major reasons why *Enterococci* survived in hospital setting (nosocomial infections) is their intrinsic resistance to many commonly used antibiotics and their ability of acquiring resistance to most of the currently available antibiotics, either due to mutation or may be by reciept of genetic material by transfer of Plasmids & transposons[48].

Antimicrobial resistance can be either inherent or acquired[14]

INHERENT OR INTRINSIC PROPERTY

The term intrinsic resistance is used to denote resistance characteristic shared by whole species exhibited in almost all the strains of that species. The genes for intrinsic resistance reside on chromosomes.

The various intrinsic traits expressed by *Enterococci* are:

- Resistance to β-Iactams, semisynthetic penicillinase resistant penicillins, cephalosporins
- Resistance to low levels of aminoglycosides
- Resistance to low levels of clindamycin.[14]

β – Lactams

The reason for intrinsic resistance to penicillin is due to the over-production of low affinity penicillin binding protein (PBP)[14,48]. The low affinity binding protein usually involved is PBP-5 for penicillin, ampicillin and other β lactams, including cephalosporins. The MIC is 2-8μg /ml for pencillin in *E.faecalis* and 16-32 μg/mg in *E.faecium*. Enterococci are moderately susceptible to ticarcillin and carbenicillin and are resistant to penicillinase resistant semisynthetic penicillins and cephalosporins. Imepenem is more active against *E.faecalis* but are bacteriostastic.

Aminoglycosides

Resistance is predominantly due to the presence of the inactivating enzyme, 2''- phosphotransferase-6''-acetyl transferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin and kanamycin. Hence resistance to gentamicin

may be a good predictor of resistance to other aminoglycosides except streptomycin where resistance is due to adenyl transferase (ribosomal resistance)[14].

MIC for streptomycin is 250 μgm/ml and for gentamicin and tobramycin 8 μg/ml to 64 μg/ml. MICs of aminoglycosides like, kanamycin, tobramycin, netilmicin for *E.faecium* are higher than other *Enterococcal* species. *E.faecium* has a chromosomally encoded acetyl transferase that may present the resistance to all amino glycosides .The synergistic activity of Pencillin and Vancomycin along with Aminoglycosides is due the increased uptake of these agents in the presence of cell wall synthesis inhibitors.

HLGR has the same mechanism but it is acquired by transmissible plasmids.

Clindamycins

Another important feature of enterococci is their resistance to clindamycin and lincomycin. MICs for most strains are 12.5 to 100 μgm/ml. .[14]

ACQUIRED RESISTANCE

This resistance results from either a mutation in existing genes or insertion of new genes. Acquired resistances are to the following drugs:

Chloramphenical, Erythromycin, Tetracyclines, High levels of clindamycin, High levels of aminoglycosides, Penicillin by means of penicillinase, Fluoroquinolones, Vancomycin

Fluoroquinolones resistance may to be due to mutation while resistance to other agents listed above can occur by acquisition of new DNA (transposons, plasmids)[14,23].

This acquisition of new DNA occurs by transduction or conjugation and the mechanisms of such acquisitions are

Broad host range plasmids, Here transfer occurs among *Enterococci* or with species of *Streptococcus, Staphylococcus, Bacillus subtilis* and others. These plasmids may be the reason for sharing of resistance genes between the *Staphylococci* and *Enterococci*.[14, 23]

Narrow host range plasmids, Here, the transfer is found only in *E. faecalis* so far. These plasmids respond to pheromones, from recipient cells, by producing aggregation substance. This substance causes characteristic and grossly appearing clumping between donors and recipient cells[14,25]

Conjugative transposons, The test-studied transposon is Tn 916, which mediates tetracycline resistance[14].

PENICILLIN RESISTANCE WITHOUT B-LACTAMASE

Existence of *Enterococci* with incresed penicillin resistance with MIC >25μgm/ml may be because of similar but increased intrinsic resistance or due to low affinity pencillin binding proteins or may be because of acquired resistance.

HIGH LEVEL AMINOGLYCOSIDE RESISTANCE (HLAR)[14]

This Plasmid mediated resistance is due to enzyme 2 phosphotransferase and 6' adenyl transferase. Resistance to gentamicin is due to the activity of 3' phosphotransferase and results in, resistance to all other aminoglycosides except streptomycin. Streptomycin resistance is due to the activity of adenyl transferase. Hence Gentamicin and Streptomycin should be tested individually to predict the resistance to aminoglycosides.

Two types of Resistance mechanisms

a) Low permeability to aminoglycosides causes moderate level resistance (MIC 64-500μg/ml), It is surpassed by synergism with cell wall active agents and effective in treatment strategy.

b) Inactivating enzymes production like acetyl transferase and adenyl transferase and also ribosomally mediated resistance causes high level resistance (MIC - 2,000μg /ml).

Resistance to streptomycin - Altered ribosomal target and modifying enzymes

Resistance to gentamicin – Mostly by modifying enzymes.

Resistance to either cell wall active agent or aminoglycoside both may lead to acquiring resistance to the synergistic killing of *Enterococci* when used in combination therapy and therefore poses a major therapeutic problem. Hence earlier detection of resistance to both these agents is significantl for the assessing the synergy effect of the combination therapy

Screening Tests

Following are the various screening tests that are recommended and available for detecting High Level Aminoglycoside Resistance.

Agar Screening Method – Murray et al. used heavy inoculums streaking onto the brain heart infusion agar containing 1000 μg of gentamicin and 2000 μg of streptomycin per ml. They observed growth on the plate corresponds with high-level resistance among the strains.

High content discs method: High content disc to identify HLAR. Gentamicin (120 μg) and streptomycin (300 μg) used. The zone of inhibition is interpreted according to CLSI 2015 guidelines.

Determination of MIC: By Broth dilution Method or by E-strip method.

VANCOMYCIN RESISTANT *ENTEROCOCCI* (VRE)

Vancomycin-resistant *E.faecalis* and *E. faecium* were reported first in 1988 in England. Emergence of *Enterococcal* resistance to vancomycin is one of the most alarming news in history of microbiology. It is reported to be inducible resistance and can be transferable by conjugation.

Vancomycin is one of the two glycopeptides currently in use to treat *Enterococcal* infections, the other one being Teicoplanin. Vancomycin resistance can be divided into high level (MIC ≥ 64 mg/ml) and low level (MIC ≥32 mg/ml) and there are various phenotypes.

The mechanism of action of Vancomycin is inhibition of transglycolysation and transpeptidation of the pentapeptide units – (the last step in peptidoglycan synthesis) and thereby interfering with the synthesis of cell wall in bacteria[29].

Based on phenotypic and genotypic characteristics they are classified s as 7 types of glycopeptides resistance – vanA, vanB, vanC, vanD, vanE, vanG & vanL[23,28]. Some of them even have subtypes also. The most clinically related phenotypes are vanA and vanB and they are generally related with *E.faecalis and E.faecium* and vanC is intrinsic to *E.casseliflavus & E.gallinarum*[23].

The genotype explains the gene clusters which encode the enzymes that are concerned in the generation of their structural components. These are the diverse peptidoglycan precursors that have reduced binding affinity for glycopeptides (vancomycin, teicoplanin or both). Phenotypic resistance may be the result of these alteration in structural components. The glycopeptide- susceptible strains have, "Dalanyl- D-alanine" depsipeptide in their cell wall, as the terminal end of peptidoglycan side chains. The antibiotic inhibits the cell wall synthesis by binding to this depsipeptide. In glycopeptide – resistant strains, this depsipeptide is replaced with "D-alanyl-D-lactate" (vanA, vanB, vanD) or "D-alanyl-Dserine" (van E, vanG) [23]. Nine different types of glycopeptide resistance operons (vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM, and vanN) have been expressed in *Enterococci* of which vanA, vanB are the most clinically important[51,52].

vanA –encoded by van A gene. It is an inducible high level resistance to both vancomycin (MIC 64-1,000 μg/ml) and teicoplanin (MIC 16-512μg/ml)[23,48,51]. It is located on the transposon *Tn1546*[51,53]. The altered gene product is "D-alanine-D-lactate". It is frequently encountered in *E.faecalis, E.faecium*

vanB –Encode by van B gene. It is an acquired inducible, resistance (variable level) to vancomycin (MIC 4-1,024μg/ml), but susceptible to teicoplanin (MIC 0.5-1μg/ml)[51,23]. The gene is mediated by trasposons *Tn1547, Tn1549, Tn5382 and they are located in plasmid*[51]. The gene product is "D-alanine-Dlactate". It has 3 subtypes (vanB1-B3) and is distributed in *E.faecalis, E.faecium.*

vanC- It is Encoded by constitutive expression of van C gene, which is located in chromosome and exhibits intrinsic low level vancomycin resistance (MIC 2-

32μg/ml) and susceptible to teicoplanin (MIC 0.5-1μg/ml)[23,51]. The end product is "D-alanine-D-serine" and has 4 subtypes dispersed in *E.gallinarum* (C1) and *E.casseliflavus* (C2-C4).

vanD – It is encoded by constitutive expression of van D gene, mediated by chromosome, moderate level vancomycin resistance (MIC 64-128μg /ml) and to teicoplanin (susceptible / resistant) (MIC 4-64μg /ml) . The product is "D-alanine-D-lactate" and is commonly found in *E.faecium and rarely in E.faecalis, E.avium* and *E.gallinarum.*

vanE– They are encoded by van E gene which is located in chromosome, results in inducible low level vancomycin resistance (MIC 16 μg/ml) , susceptible to teicoplanin (MIC 0.5μg/ml). "D-alanine-D-serine" is the end product and is found in *E.faecalis.*

vanG– They are encoded by van G gene, located in chromosome, results in inducible low level resistance to Vancomycin (MIC 16 μg/ml), susceptible to teicoplanin (MIC 0.5μg/ml). "D-alanine-D-serine" is the end product and is found in *E.faecalis.*

The identification of the different genotypes is crucial for therapeutic and infection control purposes.

Vancomycin Dependent *Enterococci*: These were first expressed in 1994 that some strains of *Enterococci* grow well only in presence of Vancomycin. If vancomycin is not present VRE retain their ability to make D-alanine-D-alanine depsipeptide and they proceed to grow normally. VDE strains are unable to make this D-alanine-D-alanine depsipeptide. Hence the presence of vancomycin provide

these strains a chance to use D-alanine-D-lactate as cell wall constituent. VDE strains are from both *E.faecalis*, *E.faecium* but relatively uncommon[23,54.]

Vancomycin Variable *E.faecium*: Vancomycin susceptible *E.faecium* following drug exposure to Vancomycin becoming resistant. They potentially posing significant challenges in both clinical as well as microbiological aspects[55].

RISK FACTORS ASSOCIATED WITH VRE:

The risk factors related with colonization and infection with VRE have been studied. The various risk factors are as follows[27,30,48,56,57]:

- Presence of immunosuppression(bone marrow transplantation/ haematologic malignancy)
- Presence of co-morbid conditions like diabetes, high APACHE (Acute Physiology and Chronic Health Evaluation) score, renal failure, malignancy.
- Prolonged stay in hospital and hospital transfer within the hospital.
- Residence in a long term care facility, contact with another infected / colonized patient.
- Previous exposure to broadspectrum antibiotics like cephalosporins, vancomycin.
- Invasive procedures, use of enteral tube feeding / sucralfate
- Contaminated medical equipment Exposure.
- Exposure to heath care personnel providing nursing to a known VRE patient.

COLONIZATION AND INFECTION:

Colonization of the gastro intestinal tract seems to be the first step in process of infection. In the majority of occasion, isolationof VRE is mostly from colonized patients rather than infected individuals. It is expressed in such a way that for every infected patient there could be around 10 colonizers[48]. Usually colonization involves gastrointestinal tract, perineal skin and rarely oral cavity and other sites. The sites involved in VRE infections are bloodstream, surgical wounds, intra-abdominal sites, intravascular catheters, prosthetic devices, and urinary tract[48]. VRE infections usually occur in debilitated and critically ill hospitalized patients. In their prior studies many authors have reported mortality rate ranging from 46%-70% among patients with VRE infetions[48]. The mortality seems to be higher in patients with prolonged VRE bacteremia such as neutropenic, liver transplant recipients and patients with chronic renal failure. Differentiation between colonization and infection, is difficult, as often these infections are polymicrobial and are isolated along with various known pathogens[48].

SOURCE OF INFECTION AND TRANSMISSION OF VRE:

The source of infection could be Endogenous or Exogenous. Endogenous from patients own gastrointestinal tract from prior colonized individuals. Exogenous from contaminated environmental surfaces and contaminated medical devices – linen,bed rails, bed pans, doorknobs, glucosemeters, IV pumps, ECG monitor,blood pressure cuffs, and stethoscope. VRE are resistant to dessication and extreme temperatures and hence persists for days to months. Contaminated food products act as a reservoir in non hospitalized individuals.

The transmission of VRE is through the contaminated hands of healthcare workers in hospital settings (nosocomial VRE infections) and coming in contact with contaminated surfaces and contaminated equipments[48,57]

SCREENING METHODS:

Disc diffusion method:

To a lawn culture of *Enterococci* (0.5 Macfarland standard) Vancomycin 30µg disc applied in 5% MHA blood agar and interpreted according to the CLSI 2015.

Agar Screening Method

Presumptive identification of VRE is done by vancomycin screen agar containing 6µg of vancomycin per ml of media.

Detection of minimum inhibitory concentration (MIC)

Confirmation is always by determination of minimum inhibitory concentration (MIC) of vancomycin and teicoplanin for the suspected VRE isolates. Broth dilution method or agar dilution method followed. Epsilometer tests and automated method for detection of MIC and interpreted according to CLSI guidelines 2015.

Agar Dilution Method: Mueller Hinton Agar was supplemented with various concentration of Vancomycin and overnight bacterial culture isolate was adjusted to 0.5 Mcfarland turbidity standard. 10µl of culture isolate was spot inoculated onto the plates. Then the culture plates were incubated for 24 hours at 37ºC and observed for growth. MIC was taken as minimum concentration of Vancomycin that inhibit growth of *Enterococci*.

Enterococci MIC for Vancomycin interpreted as follows:

≥32µg/ml - Resistant, 8-16µg/ml- Intermediately Resistant and ≤4µg/ml-Sensitive

E-test: Epsilometer test strip applied on to lawn culture of *Enterococci* in Mueller Hinton agar and incubated at 37ºC for 18 -24 hrs examine it. MIC ≤4µg/ml considered as Sensitive, 8-16µg/ml taken as Intermediately Resistant and ≥32µg/ml considered as resistant as per CLSI guidelines 2015.

Molecular methods

Polymerase chain reaction for the amplification of genes of vancomycin resistance - vanA, vanB and vanC, either conventional or multiplex PCR or by DNA hybridization probes. Use of Cepheid Expert vanA/vanB assay on inoculated enriched broths with adjusted threshold value for PCR positivity can be considered a useful tool for detection of vanA/vanB[58]

Treatment of VRE infections:

The therapeutic options for serious VRE infections are synergistic therapy with cell wall active agents high dose (ampicillin) and an aminoglycoside (provided no acquired resistance seen for both of these agents) . Other therapeutic options are as follows.

Linezolid – It is a bacteriostatic drug, belongs to oxazolindones it is FDA approved drug for treatment of VRE infections of *E.faecalis, E.faecium*. It binds to the ribosomal peptidyl transferase center and stops bacterial growth by protein synthesis inhibition[53]. It is recommended only as an alternative to other agents, play

a vital role in the endovascular infections and in meningitis[27]. Also effective in MRSE, MRSA, VISA and VRSA.

Quinpristin-Dalfopristin – It is a bacteriostatic drug, parentral semisynthetic streptogramin type A and B. it is FDA approved and active against *E.faecium* only and not against *E.faecalis*[27]. It is because of presence of an ATP binding cassette (ABC) protein homologue designated Lsa, which is likely to act as an efflux pump for this compound[29]. Mechanism of action is it binds to 50 S ribosomal subunit and blocks the translation. Effective in treatment of MRSA, MRSE, VRSA & VRE (*E.faecium* only)[53.]

Daptomycin- It is a cylic lipopeptide active against both *E.faecalis* and *E.faecium*, it is not FDA approved for VRE treatment in *E.faecium*[29]. It causes rapid membrane polarization triggering calcium dependent rapid efflux of potassium ions and thereby inhibiting RNA, DNA and Protein synthesis[53]. It is approved for VSE skin and soft tissue infections[59]. Only in therapeutic failure of the commonly used other agents it can be used. Also can be used in MRSA & VRE peritonitis[53,61].

Tigecycline-It is a glycylcycline It is FDA approved for treatment of abdominal infection and complicated skin and soft tissue infections (CSSSI's) and complicated intra abdominal infections.[29,53,60]. Binds to 30S ribosome, blocks entry of transfer RNA and prevents protein synthesis by delaying incorpoaration of aminocids into peptide chains. Also can be used in treating MRSE, VRE, Meropenem resistant *Klebsiella, Acinetobacter* and ESBL producing *Escherichia coli.*

Ortivancin- It is a Lipoglycopeptide, recently approved by FDA, used in skin and soft tissue infections, showed good in vitro activity has a promising role in

treatment of VRE in future[27,31]. They disrupt cell wall synthesis and cell membrane integrity. It is useful to treat MRSA, VISA, VRSA and VRE[53].

Tedizolid- It is a Oxazolidinones approved recently by FDA to treat acute bacterial skin & soft tissue infections. It showed good in vitro activity against VRE[31].

MULTI DRUG RESISTANCE ENTEROCOCCI:

In recent years, some strains have acquired high level resistance to multiple antibiotics including aminoglycosides, ampicillin and vancomycin. Vancomycin resistance is of particular concern because of treatment difficulties and because of the potential for this plasmid mediated vancomycin resistance trait to be transferred to other microorganisms. In the event methicillin resistant S. aureus were to acquire vancomycin resistance genes from Enterococci, this pathogen would become virtually untreatable with the current antibiotics [61]. In recent studies it has been observed that drugs which are used in treatment of VRE, like Linezolid, Daptomycin, Quinipristin / Dalfopristin, Tigecycline also has developed resistance.

PREVENTION AND CONTROL MEASURES:

CDC - Hospital Infection control practices advisory committee has given certain guidelines and recommendations to be followed for the prevention of spread of VRE

- Inappropriate use of vancomycin being a major risk factor for VRE infection and its colonization and also for emergence of vancomycin resistant *Staphylococci*. Hence prudent use of vancomycin is recommended.
- All health care personnel should be educated about the acceptable or appropriate use of vancomycin (MRSA treatment, implantation of prosthetic

devices following major surgical procedures, severe antibiotic associated colitis as a second line agent)

- Awareness for standard precautions **(**PPE), should be created among all health care workers. Standard precautions are the basic infection control precautions which should be followed in any setting where health care is delivered and are used to prevent infection from the health care workers to patients and vice versa.
- Education of the Health care personnel and patient care givers about the impact and epidemiology of VRE infections.
- For early detection of colonization of VRE effective implementation of surveillance procedures (feces cultures) should be done.
- Infection control procedures are aimed to limit cross contamination, isolation of those known VRE colonizers and patients and very strict adherence to hand washing as it's alone can slow down the spread and transmission of VRE among hospital setting to a considerable extent.
- Antibiotic stewardship programs to be conducted and awareness to be spread to strictly adopt antibiotic policy which advocates the rational use of antibiotics.

MATERIALS AND METHODS:

The present study was conducted in Department of Microbiology in Government Kilpauk Medical College and Hospital, Chennai, India.

Study Population : Patients attending outpatient department and inpatients of Govt. Kilpauk Medical College and Hospital, Chennai.

Study Period : January 2015 to December 2015 - one year

Study Design : Cross sectional study

A total of 240 *Enterococci* isolates recovered from various clinical specimens were processed for further study. Those clinical specimens such as urine, pus, blood, High vaginal swab, tissue fluids and feces were obtained from both sexes in all age groups of patients submitted to the microbiology laboratory and were analyzed for further study

The study was approved by our Institutional Ethical Committee.

INCLUSION CRITERIA :

All non duplicate *Enterococci* isolates taken from various clinical samples like urine, pus, blood, high vaginal swab, tissue fluids, faeces.

EXCLUSION CRITERIA:

Isolates other than *Enterococci* isolated from various clinical samples

METHODOLOGY

All specimen brought were subjected to the following

Direct microscopy[62]

Smears were made from all specimen except the blood. Gram staining was done. Observed for the presence of pus cells and Gram positive cocci, occurring in ovoid pairs or in short chains.

Culture

The specimens were inoculated on to Blood agar, Nutrient agar and MacConkey agar.[36,63,64,65]. They were also inoculated into Bile esculin agar medium[36,66]. Urine specimen was processed for semiquantitative culture[65]. It was inoculated onto the Cystine lactose electrolyte deficient (CLED) media [65,67]. Media containing plates after inoculation were incubated and looked for growth. They were incubated at 37°C for overnight.

Plates were examined for:

- Blood Agar : Presence of small, nonhemolytic or α hemolytic colonies
- Nutrient Agar : Presence of small, 0.5-1mm colonies
- MacConkey agar : Presence of deep pink magenta colored colonies
- Bile Esculin Agar : Blackish discoloration of the medium
- CLED media : Small, yellow colored colonies

In case of urine sample, a semi-quantitative calibrated loop technique was used to isolate the organism. One loopful holding 0.01 ml of clean catch midstream urine sample that was not centrifuged was inoculated onto the surface of Cysteine Lactose Electrolyte Deficient agar. The culture plates were incubated under aerobic conditions for 18-24 hours at 37^0 C.

Using colony counter the colonies was counted and the numbers of colony forming units were multiplied by 100 to find out the number of microorganisms present per millilitre of urine. Only the significant counts were processed in which showed 10^5 cfu/ml[68,69]. Lower counts were processed based on the clinical history of patient. The suspected *Enterococcal* isolates grown on the primary plating media such as blood agar and MacConkey agar were selected for further identification.

Following tests are done for Presumptive identification:

Colony smear[36,64,66,70]:

Gram staining was done with a smear prepared from the colonies and observed for the presence of Gram positve cocci arranged in pairs and short chain[16,18,36]. Gram Staining was done along with the Controls

Positive control : *Staphylococci* ATCC 25923

Negative control : *Escherachia coli* ATCC 25922

It is again subcultured onto nutrient agar for catalase test and blood agar for demonstration of haemolysis.

Catalase test [18,36,64,70]:

A part of a colony was taken with the help of small glass rod and immersed in a test tube containing 3% hydrogen peroxide. The catalase test was done along with controls. A rapid and sustained effervescense due to the presence of nascent oxygen was taken as positive result[26].

Positive control : *Staphylococcus aureus* ATCC 25923

Negative control : *Enterococcus faecalis* ATCC 29212

Enterococci are catalase negative or pseudocatalase positive[16,18,36]

Bile Esculin test[14,37,65,66,70]**:**

The isolates were inoculated on to the bile esculin agar and incubated at 37°C for 24 -48 hrs. A positive test was indicated by the growth of black coloured colonies indicate a positive test. It was due to hydrolysis of esculin to aesculetin which then reacts with ferric ions to form black colonies.

Heat tolerance test[14,37,65,70]**:**

Enterococcal isolates were tested for heat tolerance along with the control strains. After inoculating the isolates into BHI broth, those isolates were incubated at 60ºC for 30 minutes in a water bath. From the BHI broth subcultures were done on both blood agar and MacConkey agar prior to incubation and at intervals of 10 min, 20min and 30 minutes after incubation. Positive control used was ATCC *E.faecalis* 29212. Before reading other isolates, growth of the positive control was checked. The positive control strain has showed growth before and after heating the broth at 60ºC for 30 minutes. Similarly those isolates which showed growth before and after 30min of incubation at 60ºC were considered as heat tolerant *Enterococcal* isolates.

PYR Test[14,37,65,66,70,71]**:**

The colonies of the *Enterococci* isolates were inoculated in PYR broth **(**α - pyrrolidonyl β- naphthylamide**)** and incubated at 37^{0}C for 4 hrs. A drop of PYR reagent which is p-dimethylamino cinnamaldehyde is added to the broth. The production of cherry red colour was taken as positve. *Enterococci* are PYR **(**α - pyrrolidonyl β- naphthylamide**)** Positive.

Salt tolerance test[14,36,37,41,65,66,70,71]**:**

In brain heart infusion broth along with 6.5% Nacl, 1-2 colonies of *Enterococci* isolates were inoculated and incubated for 18-24 hrs at 37^0C. 1 % Bromothymol blue (0.002%) was used as indicator. Positive test was indicated by the presence of turbidity in the broth & colour of the broth changed to yellow.

Growth at 10° C & 45° C [14,17,37,41]**:**

In brain heart infusion broth, 1-2 colonies of *Enterococci* isolates were inoculated and incubated for 18-24 at 10° C & 45° C. After incubation the tubes were examined for growth by rotating them in front of an incandescent lamp.

Growth at pH 9.6 [14,17,36,41]**:**

Examined for growth at pH 9.6. All pH measurements were conducted with a pH meter

Litmus Milk Decolorisation test[17,41]

A heavy inoculam of the test organisim taken in a tube containing litmus milk and incubating it at 35-37^oC for 4 hours. A production of white or pale yellow colour is taken as positive and No change or pink colour is taken as negative.

Salt tolerant, Bile esculin agar positive isolates, which were able to grow on MacConkey agar and at temperatures of > 45ºC, at 10& 45ºC grown at pH 9.6 were identified as *Enterococci* and selected for further speciation[6,10].

SPECIATION OF ENTEROCOCCI

Enterococcal strains were further identified to the species level by using conventional tests designed by Facklam and Collins[36].

Sugar fermentation

Each strain of *Enterococci* were tested for its ability to ferment sugar[16,17,33,36,37,41]. 1% sugars Mannitol, Sorbitol, Sucrose, Arabinose, Raffinose, Sorbose, Lactose are added in Brain heart infusion broth with indicator bromothymol blue (0.002%). To each sugar tubes 1-2 drops 18-24 hrs old broth culture of enterococci are inoculated with the help of Pasteur pipette and incubated for 18-24 hrs at 37^0C. It's observed daily and kept for a period of 7 days. Fermentation is indicated by the colour change from blue to yellow[26]

Arginine hydrolysis[36,65,70]:

It was tested by inoculating the *Enterococci* isolates into a tube of Moeller's decarboxylase broth containing arginine and a control tube (without arginine). It was overlaid with sterile liquid paraffin and incubated at 37ºC for seven days. Anaerobic enviroment will be provided by overlaying liquid paraffin. Initially tube changed to yellow in colour and if arginine is dihydrolysed (positive test) the tube with arginine reverts back to the original purple colour due to alkalinization, Negative test is indicated by persistant yellow color.

Positive control : *Enterococcus faecalis* ATCC 29212 (purple colour)

Negative control : *Klebsiella Pneumoniae* (yellow colour)

Pyruvate fermentation[17,36] :

Enterococci species first grown in brain heart infusion broth from which 1 – 2 drops was then inoculated into pyruvate broth and incubated at 37°C for 18-24 hrs. Bromothymol blue (0.002%) is used as indicator. Positive test was

indicated by the colour change from blue to yellow indicating production of acid due to pyruvate fermentation.

Motility[36,65,70]**:**

The brain heart infusion broth culture of *Enterococci* are observed for motility by hanging drop preparation (*E.casseliflavus* and *E.gallinarum* are motile)

Pigment Production[36,65,70]**:**

Enterococci species is streaked on Tryptose soya agar and incubated at 37 degrees for 24 -48 hrs to look for yellow pigment production[70]. A cotton swab was used to pick up the colonies and was examined for yellow colour formation. (*E.casseliflavus* & *E.munditi*) produce pigment

The additional tests performed were production of black coloured colonies on 0.04% tellurite agar, which is a feature of *E.faecalis*[4,17,66,70,72].

Characteristics of *E.faecalis* and *E.faecium*

CHARACTERISTICS	***E.faecalis***	***E.faecium***
Gram stain	Gram positive cocci occurring in pairs and short chains	Gram positive cocci occurring in pairs and short chains
Catalase	Negative	Negative
Motility	Non motile	Non motile
Blood agar (5% SBA)	α hemolytic or non hemolytic	α hemolytic or non hemolytic
MacConkey agar	Magenta coloured colonies	Magenta coloured colonies

CHARACTERISTICS	*E.faecalis*	*E.faecium*
Bile esculin agar	hydrolyses esculin and causes blackening of the medium.	hydrolyses esculin and causes blackening of the medium.
Heat tolerance	Survived at a temperature of 60ºc for 30 minutes.	Survived at a temperature of 60ºc for 30 minutes.
Salt tolerance	Survived in 6.5%NaCl	Survived in 6.5%NaCl
Pigment production	No Pigment production.	No Pigment production.
Arginine dihydrolysis	Arginine hydrolysed, produces Deep purple colour	Arginine hydrolysed, produces Deep purple colour
0.04% tellurite agar	Black coloured colonies produced.	-
Carbohydrate utilization (1%)	Pyruvate and Sorbitol – Fermented Arabinose,Sorbose- Not Fermented	Arabinose - fermented, Sorbose &Pyruvate – not fermented.

E.raffinosus, E.avium, E.durans, E.hirae were differentiated based on the following characteristics

Enterococcal species	**Arginine dihydrolysis**	**Fermentation of sugars**
E.raffinosus Group I.	Not hydrolysed	Mannitol, Raffinose, Arabinose, Sorbose fermented
E.avium Group I	Not hydrolysed	Mannitol , Arabinose, Sorbose fermented, Raffinose not fermented
E.durans Group III	Hydrolysed	Raffinose, Sucrose & Pyruvate not fermented.
E.hirae GroupIII.	Hydrolysed	Raffinose & Sucrose Fermented Pyruvate not fermented

Speciation of *Enterococci*

Species	Mannitol	Arabinose	Raffinose	Sorbitol	Sucrose	Pyruvate	Arginine
E.faecalis	+	-	-	-	+	+	+
E.faecium	+	+	V	-	+	-	+
E.raffinosus	+	+	+	+	+	+	-
E .avium	+	+	-	+	+	+	-
E.durans	-	-	-	-	-	-	+
E.hirae	-	-	+	-	+	-	+

DETECTION OF VIRULENCE FACTORS OF *ENTEROCOCCI*:

Haemolysin[6,67,73,74]:

The hemolysin production by *Enterococci* was tested by the plate hemolysis test. *Enterococci* was inoculated onto 5% sheep blood agar and incubated at overnight 35 degree celcius[67,73,74]. Haemolysin production is indicated by a zone of complete lysis of the RBCS around the colony with clearing of the surrounding medium[74].

Gelatinase Production[6,67,73,74]:

Gelatinase production was tested with gelatin agar. *Enterococci* was inoculated onto the plate and was incubated at 37 degree Celsius for 24 hours. A turbid halo or a zone of clearing around the colonies was considered Positive for Gelatinase production [6,73,74].

Biofilm[73,74,75]**:**

The organisms were isolated from fresh culture plates and were inoculated into Brain Heart Infusion Broth added with 2% sucrose and incubated for 24 hours at 37^0 celsius. Broth was diluted to 1: 100 with fresh medium,. A sterile polystyrene 96 wells, flat bottom culture plate was taken and to which 200 microlitres of the diluted cultures were inoculated [73,74]. Only medium without any culture was used as control in order to check the sterility and the non specific binding of the media. At 37^0 celsius for 24 hours the tissue culture plate was incubate. After the incubation period, the contents of each well were removed by tapping the plates. To remove the free floating planktonic bacteria, wells were washed for 4 times using 0.2 ml of phosphate buffer saline. Following which fixation was done with 2% sodium acetate to fix the biofilms formed within the plate. Again it was stained for one minute with 0.1% crystal violet. Thorough washing with deionised water is important to remove excess stain and plate was kept for drying. If the organisms were adherent, they usually form biofilm on all the sides of the well and with crystal violet were uniformly stained. At a wavelength of 570 nm using micro ELISA auto reader, Optical density (OD) of stained bacteria were detected. OD values were considered as index of bacteria adhering to surface and the biofilm formation. Usually this procedure was done in triplicate, data averaged and the standard deviation was calculated. Usually Media control well's mean OD value was subtracted from all the test values. Those OD values >0.2, 0.081-0.2,<0.081 were considered as High, Moderate, Low biofilm producers respectively[74].

ANTIBIOTIC SUSCEPTIBILITY TESTING

Using Kirby Bauer disk diffusion method on Mueller Hinton agar, Antibiotic susceptibility testing was performed. Interpretation of the results were made by measuring the zone size of inhibition according to CLSI 2015(M100-S25) guidelines. *Enterococcus faecalis* (ATCC 29212), was used as quality control strains[76,77].

TURBIDITY STANDARD FOR INOCULAM PREPARATION [63,78,79]

For an antibiotic susceptibility test, inoculum density was standardized by a Barium sulfate(BaSO4) turbidity standard, It should be equal to 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension).

Inoculum preparation

Approximately 3-5 morphologically similar colonies must be taken and in a sterile test tube it was mixed with about 4-5 ml of a suitable broth medium. The broth culture is then incubated at 35˚C till it attains 0.5 McFarland standard turbidity. This suspension approximately represent 150 million organisms/ml.

Method of inoculation of test plates

The turbidity of the test medium was adjusted, it was inoculated within 15 minutes into the plates. A sterile cotton swab was taken and immersed into the suspension. To get rid of the excess inoculum from the swab it was pressed by the sides of the tube. A dried Mueller Hinton agar plate was taken and the swab was then streaked onto it. This procedure of streaking was repeated for 2-3 times by

rotating the plate at 60˚ so that the uniform distribution of the inoculum over the plate was possible. Finally, it was swabbed around the rim of the plate. Within 15 minutes, the antibiotic discs were dispensed onto the plate. It should be pressed firmly to achieve complete contact of the discs with the surface of the agar. It was ensured that the discs were kept at 24mm from center to center. After dispensing the drugs, within 15 minutes the plate was kept in the incubator in inverted position. The plate was then examined after overnight (16-18 hrs) incubation. If the inoculum was good, then a confluent lawn of growth will be seen and the zone of inhibition will also be seen as uniformly circular. The diameter of the inhibition zone were then calculated with the help of rulers or sliding calipers. The zone of inhibition of the bacterial growth around each disc is measured under reflected light except for Vancomycin which should be read through transmitted light. According to the CLSI guidelines 2015, the interpretation were made as susceptible, intermediate and resistant[76].

The antibiotics tested were as follows

Penicillin 10U, Ampicillin (10 µg), Erythromycin (15µg), Doxycycline (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), High level gentamicin (120µg), High level streptomycin (300µg), Nitrofurantoin (300µg), Vancomycin (30µg), Teicoplanin (30µg), Linezolid

Nitrofurantoin used only in Urinary isolates [80,81]. Erythromycin used only in other than urinary isolates[81].For VRE isolates (Both *E.faecalis* & *E.faecium*) -

Linezolid (30µg), Tigecycline (15 µg)[3]. For VRE (only *E.faecium*) – Quinpristin / Dalfopristin (15µg) [3,27].

SCREENING TESTS FOR HIGH LEVEL AMINOGLYCOSIDE RESISTANCE (HLAR)[67,68,70,81,82,83]:

The *Enterococci i*solates were screened for High level Aminoglycoside resistance by using the antibiotic discs – High level streptomycin (HLS) 300µg and High level gentamicin (HLG) 120µg[67]. On Mueller Hinton agar using McFarland 0.5 turbidity standard of bacterial suspension, streaking was made and discs were kept by standard disc diffusion method as described above and incubated overnight at 37ºC. As per CLSI standards **t**he results were interpreted susceptible 10mm, inconclusive-7-9mm, resistant – 6mm[67,76].

DETECTION OF VANCOMYCIN RESISTANCE

VANCOMYCIN SCREEN AGAR [4,67,68 ,84,85,86]:

Presumptive identification of resistance to can be done by using Vancomycin screen agar (ie) brain heart infusion (BHI) agar added with 6 µg /ml vancomycin[10B,67,85]. 10µl each of 0.5 McFarland suspension of the isolate, along with negative and positive control strains, were spot inoculated over the surface of agar and aerobically incubated at 35±2ºC for 24hrs. Presumptive resistance to vancomycin was indicated by the growth of one or more colonies. It should be followed by by determination of the Minimum inhibitory concentration (MIC) for vancomycin which is confirmatory[67,68,87].

VANCOMYCIN E TEST[6,65,68,84,88]

By Kirby bauer disc diffusion method, those *Enterococci* that were intermediate sensitive or resistant to Vancomycin were confirmed by determination of their Minimum inhibitory concentration (MIC) by E- test or Epsilometric test

Method: A lawn culture of *Enterococci* of 0.5 Mcfarland standards was done on 5% Mueller Hinton agar. By using sterile forceps, MIC (E) strip was taken and applied on to the plate in such a way that its MIC scale facing up and higher concentration facing the edge of the plate. Incubate it 18 -24 hrs at 37 degrees and examine it. Results were interpreted according to CLSI guidelines 2015 by the zone of inhibition in form of ellipse[76].

TEICOPLANIN E TEST[88]

On 5% Mueller Hinton agar, a lawn culture of *Enterococci* of 0.5 Mcfarland standards was done. MIC (E) strip was applied on to the plate by using sterile forceps. At 37 degrees it was Incubated for 18 -24. Results were interpreted according to CLSI 2015 guidelines.

Antibiotics Kept By E-strip Method	**MIC Interpretive Criteria (µg/mL)**		
	Sensitive	**Intermediate**	**Resistant**
Vancomycin	≤4	8-16	≥32
Teicoplanin	≤8	16	≥32

MIC FOR VANCOMYCIN BY AGAR DILUTION METHOD[70]

Determination of MIC of Vancomycin for *Enterococcal* isolates which grew on Vancomycin agar screen was done by Agar dilution method [67,87,89].

Procedure Included were:

1) Dilution of Antibiotics.

2) Inoculum Preparation.

1) Dilution of Antibiotics:

a) Range of dilution:

It was made according to CLSI guidelines, took two dilutions above and below the range decided. For Vancomycin to Enterococci (1- 128 µg/ml). Sensitive ≤ 4 µg/ml, Intermediate 8-16µg/ml, resistant ≥ 32µg/ml. 18 ml of Mueller Hinton agar with 2ml of antibiotic was taken. 1 ml should contain antibiotic for 10ml of media highest concentration, so needed is 128 µg/ml. Thus for 10ml it is 1280 µg/10ml.

b) Calculation of Stock Solution

Volume (ml) = [wt of antibiotic (mg) * Potency (µg / mg) / concentration (µg/ ml)] For 20ml, stock solution, 20 =[Xmg * 1000 / 1280], Hence X=25.6 mg. So, 25.6 mg of vancomycin was dissolved in 20ml of distilled water. Stock solution could be preserved for a week by storing it at 8°C and pure drug can be kept in deep freezer at - 20° C for a week.

c) Serial double dilution done with 2ml volume.

In this study dilution range (32µg/ml→ 0.25µg/ml). Then Mueller Hinton agar was cooled to 45° to 50° C after autoclaving (121 C 15lb for 15 minutes). Mueller Hinton agar 18ml was added for every dilution, mixed and poured in respective Petri dishes. All the plates along were incubated for 18hrs at 35° C. For sterility check a control plate was also incubated along with the test plates.

2) Preparation of inoculums:

To 3ml of peptone water, 5-6 colonies were inoculated and then it was incubated for 3- 8 hours at 37°C to adjust the turbidity to 0.5 Mc Farland standards (1.5x10 CFU/ml). Later 1:10, 1:100 dilutions to get 10 CFU/ml, finally 10µl of 4 diluted growth carries 10 CFU per spot inoculation to the respective plates. All test strains were inoculated with susceptible and resistant strains into various concentration of agar plates and drug free (control) plate. Incubate at 37 C for 18-20 hours.

Reporting

Confirm all the test strains along with control strains have grown on control plate (plate without the drug). Confirm whether the controls were within the normal range. If the above said things were confirmed then only reporting to be done further and MIC is validated. Minimum inhibitory concentration of the drug was taken as the lowest concentration of drug that could inhibit the growth of the organism.

Enterococci MIC for Vancomycin interpreted as follows:

≤4µg/ml-Sensitive, 8-16µg/ml- Intermediately Resistant and ≥32µg/ml - Resistant

MOLECULAR METHOD FOR THE DETECTION OF VRE[4,86,89]:

Polymerase chain reaction (PCR) assay was performed by the for the detection of genes responsible for Vancomycin resistance in *Enterococci* especially in *E.faecalis* and *E.faecium*. The DNA was extracted from the *Enterococci* isolates by using Pure Fast Bacterial Genomic DNA Mini Spin Prep Kit and subjected to PCR and using gel electrophoresis gene product were viewed.

Master Mix 2X - Constituents

1. Taq DNA Polymerase - 2Units
2. Taq reaction buffer - 10X
3. Magnesium Chloride. - 2mM
4. 10mMdNTPs mix - 1µl.
5. Polymerase Chain Reaction additives.

For Gel Electrophoresis

1. Agarose, 2. 50XTAE buffer, 3. 6Xgel loading buffer, 4. Ethidium bromide

Extraction of DNA from the *Enterococcal* isolates:

1. 1.5ml of overnight culture taken & centrifuged at 12000 rpm at 4º C for 5min.
2. To the pellet 180µl of Lysozyme digestion buffer was added

3. To this 20µl of Lysozyme was added and incubated for 10 min at 37 ºC.
4. Then 200µl of Lysis buffer was added.
5. Then20_l of proteinase K was added mixed well, incubated at 56ºC for 15 min in water bath
6. To this 200µl of Isopropanol added & mixed well by inverting several times.Whole sample was transferred to spin column using pipette & centrifuged for1min at 10,000rpm.
7. 500µl of wash buffer added to the spin column and centrifuged for 30 seconds & the flow-through was discarded, the spin column is placed back in collection tube.
8. Washing repeated twice using wash buffer II.
9. The flow- through is discarded mini spin centrifuged for 1 min to remove residual ethanol.
10. The spin column is placed in a fresh centrifuge tube and 50µl of pre warmed Elution buffer was added to the spin column, incubated for 2 min at room temperature, centrifuged for 1min.
11. Then the spin column is discarded DNA present in the centrifuge tube is stored at at -20º C.

5µl of this elute was used for PCR.

The vanA Primers designed by as follows.

Forward Primer: 5’ -TGCGCGGAATGGGAAAACGACA-3’

Reverse Primer: 5’ -CAGCCCGAAACAGCCTGCTCAA-3’

The PCR Product size is 473bp representing van A gene.

The vanB primers designed by as follows.

Forward Primer: 5’ - TCTTTGTGAAGCCGGCACGGTC -3’

Reverse Primer: 5’ - AGCCGACCTCACAGCCCGAAAT -3’

PCR Product size is 147bp representing van B gene.

An optimal negative control was employed using 1 µl molecular grade water.

PCR amplification:

The PCR reactant mixture for each sample is prepared by adding 20_l of PCR Master Mix, 2_l of Van A Gene specific Primer mix , 5_l of Purified DNA of each sample and 3_l of Nuclease free water to a total final volume of 30_l.PCR amplification was performed in thermal cycler (MyGenie, Bioneer,South Korea) using the following thermal profile which consists of one cycle of initial denaturation at 95ºC for 4min followed by 35 cycle of denaturation at 95ºC for 30sec, primer annealing at 62ºC for 30sec, extension at 72ºC – 30sec and one cycle of Final extension at 72ºC for 5min.

Analysis of PCR product was done by agarose gel electrophoresis.

About 1.5% of agarose gel was prepared by mixing 0.75 grams of agarose powder in 50 ml of electrophoresis buffer and heated in a microwave oven till agarose is uniformly dissolved. After cooling to 56ºC, 5µl of Ethidium bromide was added using gloved hands. Ethidium bromide is carcinogenic and hence should be handled with gloved hands and the tip is discarded into the black bin. After cooling the solution, it is poured into a gel casting tray containing comb and allowed to solidify. After hardening, the gel is placed in the electrophoresis tank. The electrophoresis buffer provided in the kit is diluted ten times and is poured into the

tank till the gel is completely immersed, then the comb was carefully removed. Then the electrical leads were connected to the electrophoresis tank. About 15µl of loading buffer containing the PCR product and the tracking dye is loaded into each well using micropipette. About 10µl of 100 bp DNA ladder was loaded into the first well followed by 15µl of the sample in the other wells. A constant current of 100 volts was applied and the gel is allowed to run till the tracking dye reaches three fourth of the gel. Then the gel tray is removed from the tank. Then the gel is removed from the tray and placed in the UV transilluminator for observation of bands of 473 bp and 147bp size for van A and van B primer respectively.

STATISTICAL ANALYSIS

The outcome of the study was observed, recorded and analysed. The data which were analysed were presented in the form of statistical tables, histograms and pie charts in appropriate places wherever necessary. All the data were documented and studied in detail. P values were calculated by Chi –Square test to compare the proportion between categorical variables. If expected cell frequency is less than five in more than 20% of cells then Fisher's exact Chi –Square test is applied. SPSS (Stastical package for the social science) version 22.0 is used to analyse the data. Significance level is fixed as 5% (α=0.005).The documented data were further in detail compared and discussed and with studies those having similar data & results which were published in reputed scientific journals.

RESULTS

This study was carried out in the department of Microbiology during the 12 month period from January 2015 to December 2015. The results were analyzed as follows. A total of about 240 *Enterococcal* isolates were recovered from the above samples, of which majority were from 131 urine specimens, 60 from pus specimens, 31 from blood specimens, 18 from High Vaginal swabs.

Table-1 AGEWISE *DISTRIBUTION* OF PATIENTS WITH ENTEROCOCCAL ISOLATES (n=240)

AGE GROUP (IN YEARS)	NO. OF PAIENTS WITH *ENTEROCOCCAL* ISOLATES
0-1	9(3.75%)
1-15	18(7.50%)
16-30	61(25.42%)
31-45	56(23.33%)
46-60	62(25.83%)
61-75	29(12.08%)
>75	5(2.08%)
Total	240(100%)

Among the isolates 25.83% are from 46-60 years age group followed by 25.42% in 16-30years , 23.33% in 31-45 years and 12.08% in 61-75 years ,which showed significance statistical value $p<0.001$. Out of the total 240 *Enterococci* isolated, majority were isolated from adult patients 215 (89.58%), however around 25(11.42%) of isolates from pediatric patients.

CHART - 1

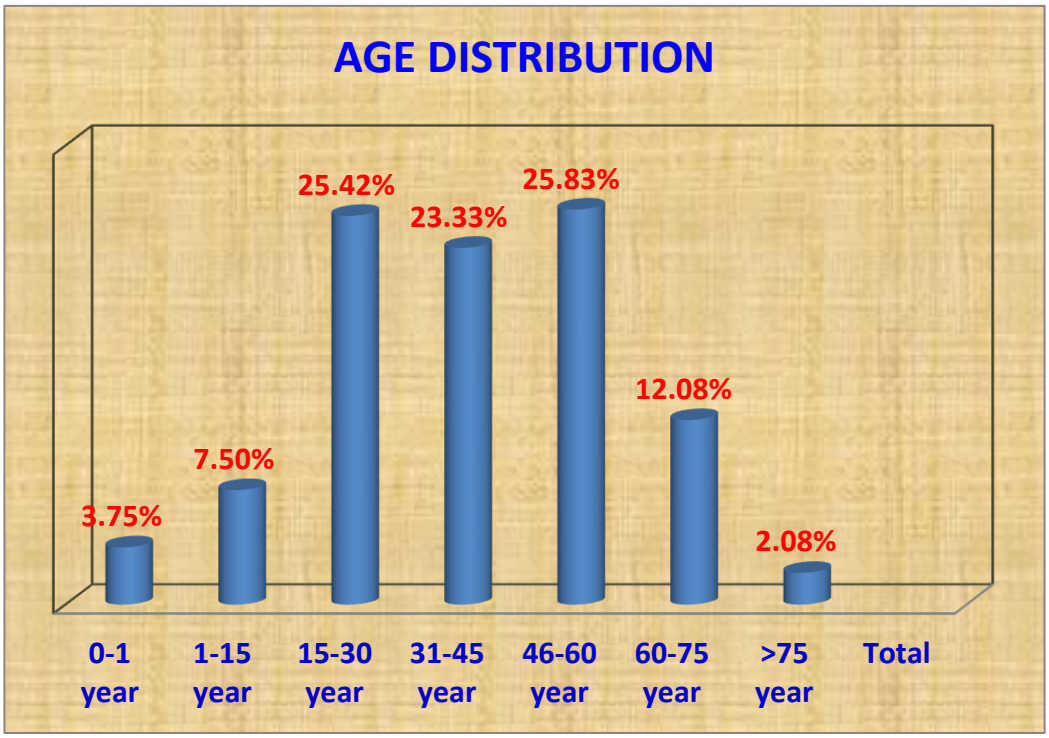


CHART -II

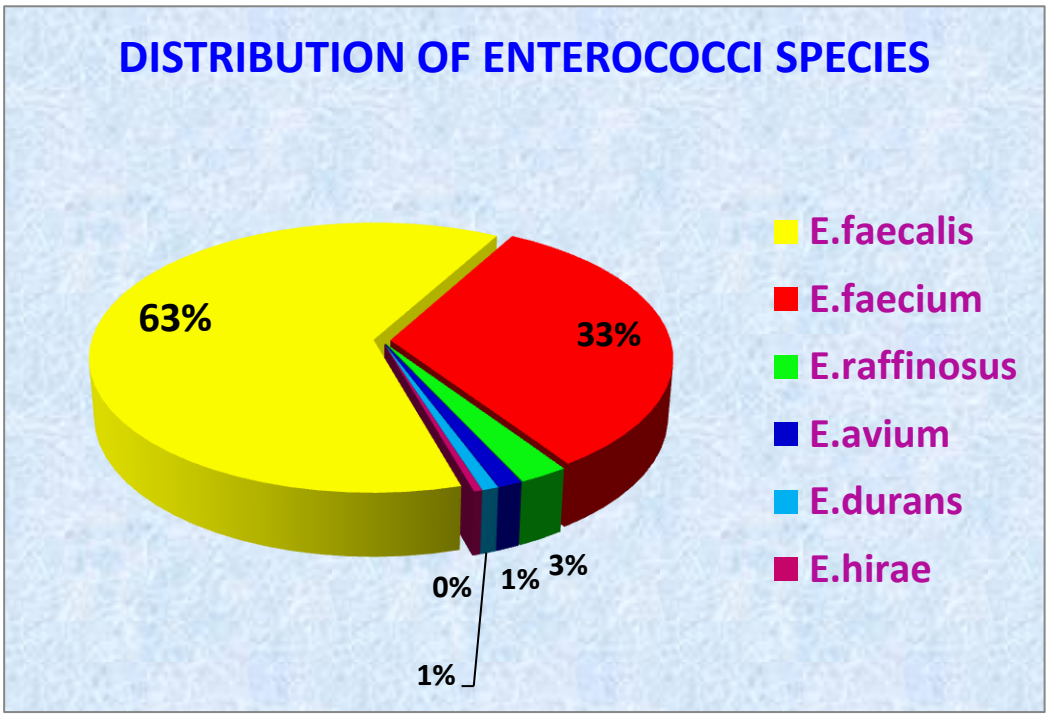


TABLE 2. SEXWISE DISTRIBUTION OF PATIENTS WITH ENTEROCOCCAL ISOLATES (n=240)

SEX	NO. OF PAIENTS WITH *ENTEROCOCCAL* ISOLATES
Male	129(53.75%)
Female	111(46.25%)
Total	240

Among 240 *Enterococcal* Isolates, Male: Female ratio is 1.17:1.

Males form 53.75% of the isolates and female 46.25% of the isolates

TABLE-3 DISTRIBUTION OF ENTEROCOCCAL ISOLATES (n=240)

ENTEROCOCCAL SPECIES	NO. OF ORGANISMS
E.faecalis	150(62.50%)
E.faecium	78(32.50%)
E.raffinosus	6(2.50%)
E.avium	3(1.25%)
E.durans	2(0.83%)
E.hirae	1(0.42%)
TOTAL	240

Out of 240 *Enterococcal* isolates, *E.faecalis* is the predominant species 150(62.50%), followed by *E.faecium* 78(32.50%) and rest contributes to 5%, which is significant as P value showed <0.001

TABLE-4 DISTRIBUTION OF ENTEROCOCCAL ISOLATES AMONG VARIOUS SPECIMENS (n=240)

***ENTEROCOCCAL* SPECIES**	**URINE**	**PUS / WOUND SWAB**	**BLOOD**	**HIGH VAGINAL SWAB**	**TOTAL NO. OF ORGANISMS**
E.faecalis (n=150)	87(66.41%)	31(51.67%)	18(58.06)	14(77.78%)	150(62.50%)
E.faecium (n=78)	36(27.48%)	26(43.33%)	12(38.71%)	4(22.22%)	78(32.50%)
E.raffinosus (n=6)	4(3.05%)	1(1.67%)	1(3.23%)	-	6(2.50%)
E.avium (n=3)	2(1.53%)	1(1.67%)	-	-	3(1.25%)
E.durans (n=2)	1(0.76%)	1(1.67%)	-	-	2(0.83%)
E.hirae (n=1)	1(0.76%)	-	-	-	1(0.42%)
TOTAL	131 (54.58%)	60 (25%)	31 (12.92%)	18 (7.50%)	240(100%)

The isolates were from Urine 131(54.58%), Pus 60(25%), Blood 31(12.92%), and High vaginal Swab 18 (7.50%)

TABLE-5 DISTRIBUTION OF ENTEROCOCCAL ISOLATES (n=240)

ENTEROCOCCAL SPECIES	**ICU**	**NON ICU (OTHER WARDS)**	**OPD**
E.faecalis	14(5.83%)	120(50%)	16(6.67%)
E.faecium	16(6.67%)	52(21.67%)	10(4.17%)
E.raffinosus	1(0.42%)	5(2.08%)	-
E.avium	-	1(0.42%%)	2(0.83%)
E.durans	-	2(0.83%)	-
E.hirae	-	-	1(0.42%)
TOTAL	31 (12.92%)	180(75%)	29(12.08%)

In ICU set up *E.faecium* is the predominant species isolated followed by *E.faecalis*, *E.raffinosus*. Isolates were more from Non ICU (75%) followed by ICU (12.92%), and OPD (12.08%), which is statistically significant as P value showed <0.001.

TABLE-6 WARD WISE DISTRIBUTION OF ENTEROCOCCAL ISOLATES IN VARIOUS CLINICAL SPECIMENS (n=240)

NAME OF THE WARD	URINE	PUS	BLOOD	HIGH VAGINAL SWAB	TOTAL ISOLATES
ICU	3	11	17	-	31(12.92%)
Medicine	28		9	-	37(15.42%)
Surgery	55	23	1	-	79(32.92%)
Pediatrics	8	2	1	-	11(4.58%)
OG	11	1	1	18	31(12.92%)
Burns	-	20	2	-	22(9.17%)
OPD	26	3	-	-	29(12.08%)
TOTAL	131	60	31	18	240(100%)

32.92% isolates were from Surgical ward followed by 15.42% from Medical Ward, 12.08% isolates were from OPD, which is statistically significant as P<0.001.

CHART - III

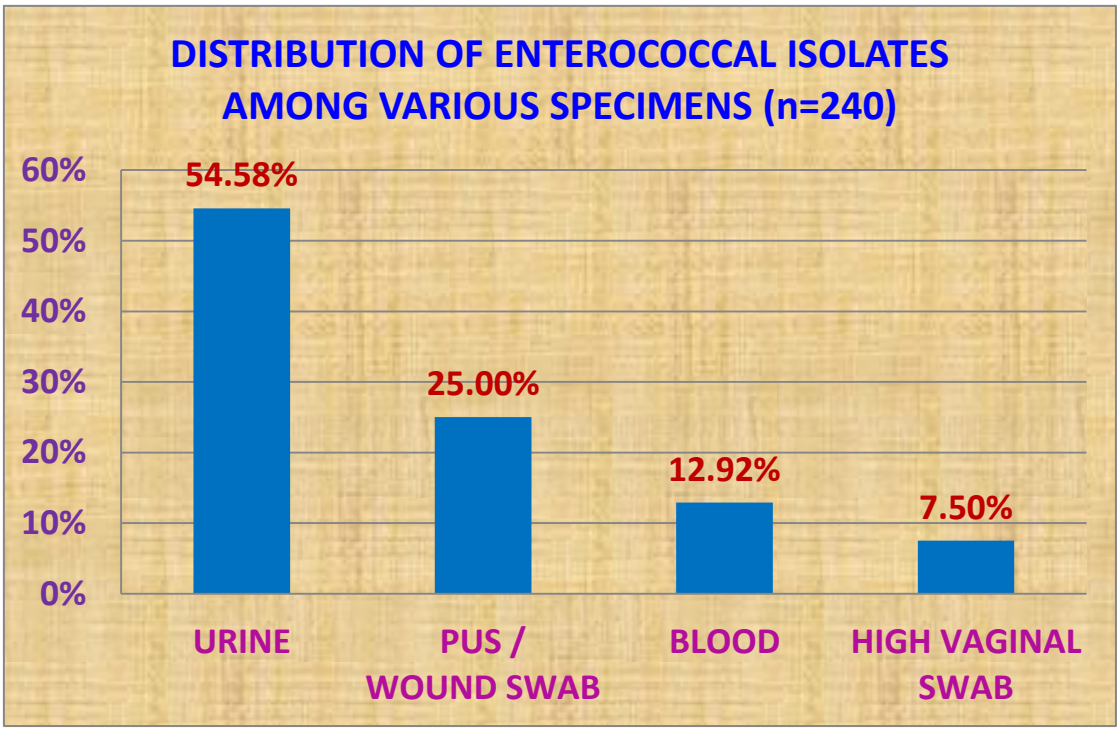


CHART – IV

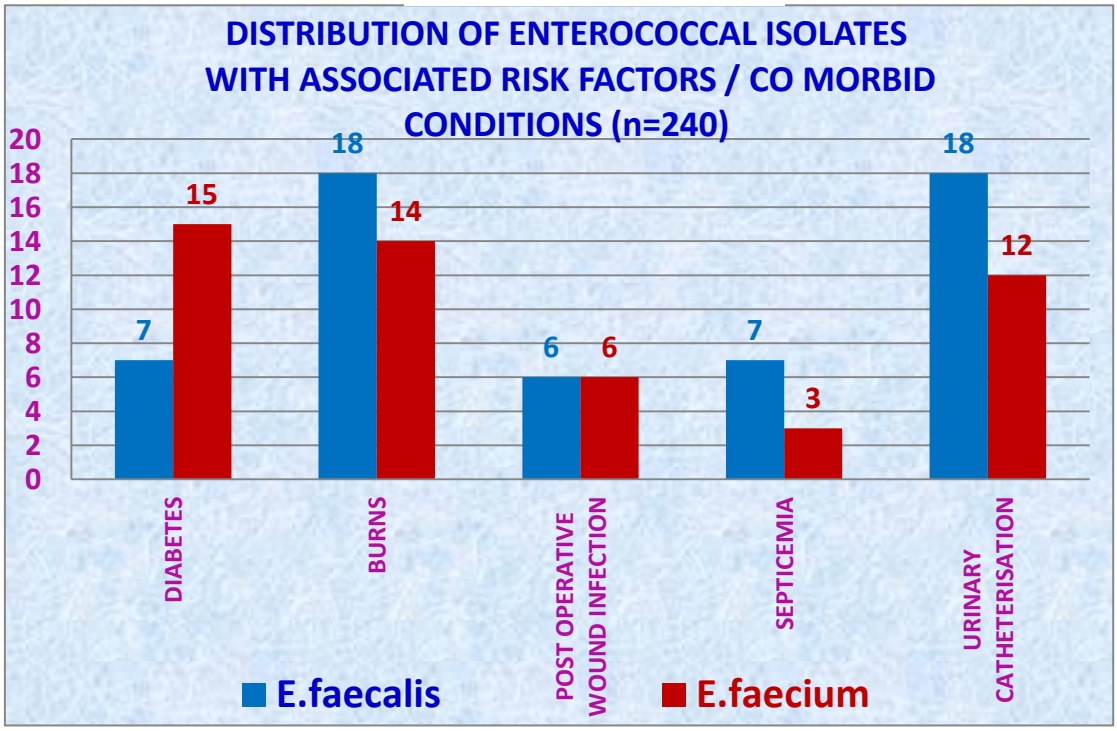


TABLE -7 DISTRIBUTION OF ENTEROCOCCAL ISOLATES WITH ASSOCIATED RISK FACTORS / CO MORBID CONDITIONS (n=240)

***ENTEROCOCCAL* SPECIES**	**URINARY CATHETERISATION**	**BURNS**	**DIABETES**	**POST OPERATIVE WOUND INFECTION**	**SEPTICEMIA**
E.faecalis	18	18	7	6	7
E.faecium	12	14	15	6	3
E.raffinosus	1	-	-	-	
E.avium	-	-	1	-	
E.durans	-	1	-	-	
E.hirae	-	-	-	-	
TOTAL	31(12.92%)	33(13.7%)	23(9.58%)	12(5%)	10(4.17%)

Enterococci were isolated more from burns wound (13.7%) followed by Urinary catheterization (12.92%), Diabetes (9.58%), Post operative infection (5%), Septicemia (4.17%).

TABLE - 8 CORRELATION BETWEEN CATHETERISATION AND ENTEROCOCCAL INFECTION (UTI) (n=131)

***ENTEROCOCCAL* SPECIES**	**CATHETERISED URINE**	**NON CATHETERISED URINE**
E.faecalis	19(14.05%)	68(51.91%)
E.faecium	8(6.11%)	28(21.37%)
E.raffinosus	1(0.76%)	3(2.29%)
E.avium	-	2(1.53%)
E.durans	-	1(0.76%)
E.hirae	-	1(0.76%)
TOTAL	28(21.37%)	103(78.63%)

Out of the 131 Urine samples 28(21.37%) were from catheterised patients.

From the catheterised patients 19 were *E.faecalis*, 8 were *E.faecium* & 1 was *E.raffinosus*.

TABLE-9 IDENTIFICATION OF ENTEROCOCCUS SPECIES (n=240)

Biochemical Test	***E.faecalis* (n=150)**	***E.faecium* (n=78)**	***E.raffinosus* (n=6)**	***E.avium* (n=3)**	***E.durans* (n=2)**	***E.hirae* (n=1)**
Glucose	150(100%)	78(100%)	6(100%)	3(100%)	2(100%)	1(100%)
Mannitol	150(100%)	75(96.15%)	6(100%)	3(100%)	-	-
Arabinose	-	78(100%)	6(100%)	3(100%)	-	-
Raffinose	-	28(35.89%)	6(100%)	-	-	1(100%)
Sorbitol	140(100%)	44(56.41)	6(100%)	3(100%)	-	-
Sucrose	150(100%)	78(100%)	6(100%)	3(100%)	-	1(100%)
Lactose	144(96%)	78(100%)	6(100%)	2(66.67%)	2(100%)	-
Pyruvate Utilization	150(100%)	-	5(80%)	3(100%)	-	-
Arginine Decarboxylation	150(100%)	78(100%)	-	-	2(100%)	1(100%)
Tellurite Reduction	150(100%)	-	-	-	-	-

Identification of *Enterococci* to the species level was readily achieved by using the battery of biochemical tests suggested by Facklam and Collins.

TABLE-10 PRODUCTION OF VIRULENCE FACTORS AMONG ENTEROCOCCAL ISOLATES (n=240)

***ENTEROCOCCAL* SPECIES**	**HEMOLYSIN**	**GELATINASE**	**BIOFILM**
E.faecalis (n=150)	30(20%)	42(28%)	78(52%)
E.faecium (n=78)	14(18%)	20(25.64%)	33(42.30%)
TOTAL	44(18.33%)	62(25.83%)	114(47.5%)

Hemolysin, Gelatinase, Biofilm were produced by *E.faecalis* & *E.faecium*. *E.faecalis* 30(20%) showed more production of Hemolysin than *E.faecium* 14(18%). Gelatinase was produced by *E.faecalis* 42(28%), *E.faecium* 78(52%). Biofilm was produced by *E.faecalis* 78(52%), *E.faecium* 33(42.30%).

TABLE-11 PRODUCTION OF VIRULENCE FACTORS AMONG ENTEROCOCCAL ISOLATES IN PATIENTS WITH ASSOCIATED RISK FACTORS / CO MORBID CONDITIONS (n=240)

RISK FACTORS / CO MORBID CONDITIONS	**HEMOLYSIN**	**GELATINASE**	**BIOFILM**
Urinary tract Infection (n=131)	27(20.61%)	31(23.66%)	65(49.62%)
Burns (n=33)	6(18.18%)	11(33.33%)	20(60.61%)
Diabetes mellitus (n=23)	2(8.70%)	8(34.78%)	12(52.17%)
Post operative Wound Infection (n=12)	4(33.33%)	5(41.67%)	8(66.67%)
Septicemia (n=10)	3(30%)	4(30%)	6(60%)
Others (n=31)	2(6.45%)	3(12.90%)	3(9.68%)
TOTAL	44(18.33%)	62(25.83%)	114(47.5%)

CHART - V

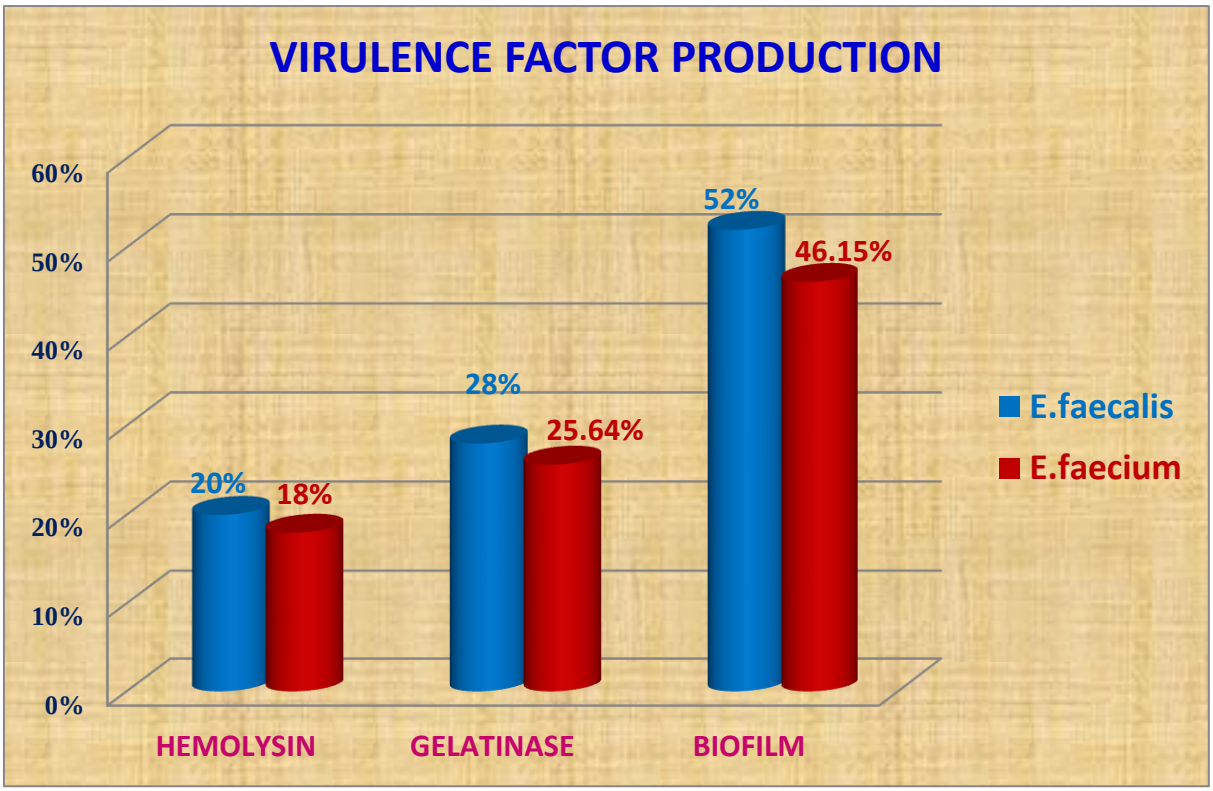
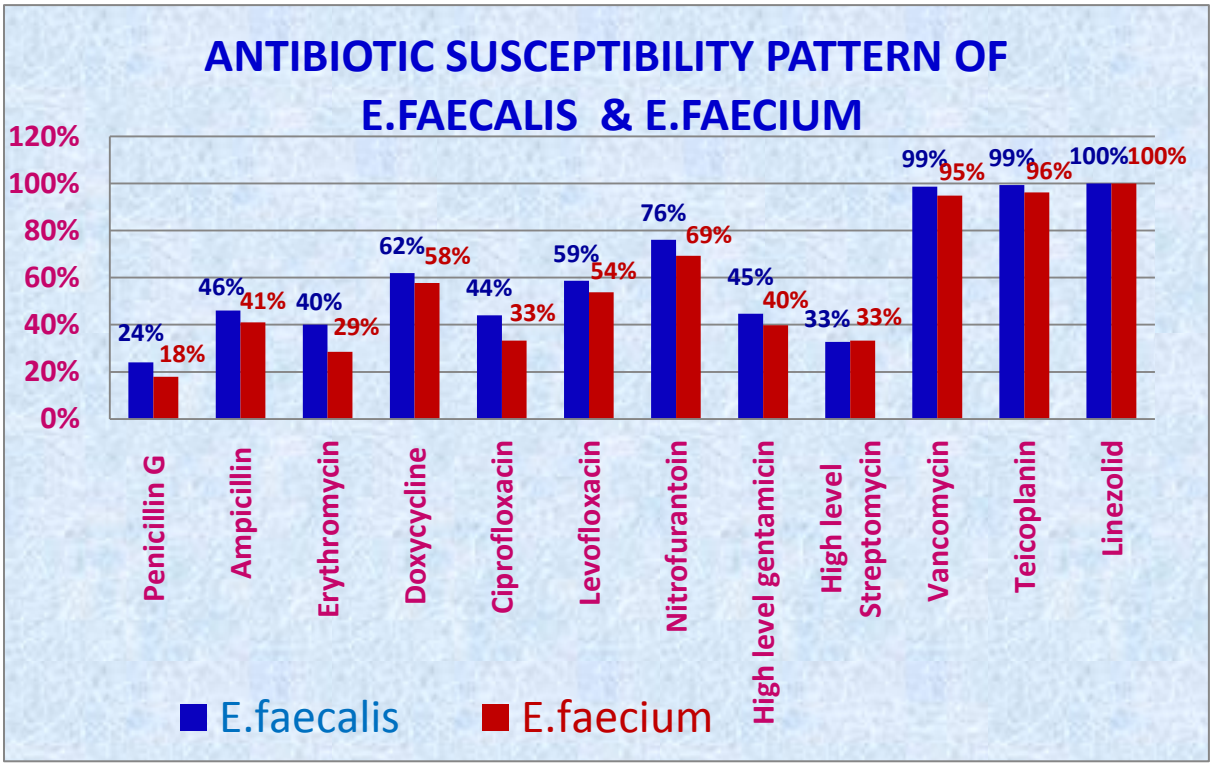


CHART - VI



Virulence factors were more produced in Post operative wound infection followed by burns wound infection, septicemia, diabetes, urinary tract infections. Hemolysin, Gelatinase, Biofilm were more produced in Post operative wound infection.

TABLE -12 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *E.FAECALIS* BY KIRBY BAUER DISC DIFFUSION METHOD (n=150)

ANTIBIOTICS	**Dosage Units**	***E.faecalis***	
		Sensitive	**Resistant**
Penicillin G	10U	36(24%)	114(76%)
Ampicillin	10 µg	69(46%)	81(54%)
Erythromycin	15 µg	25*(39.68%)	38*(60.32%)
Doxycycline	30 µg	93(62%)	57(38%)
Ciprofloxacin	5 µg	66(44%)	84(56%)
Levofloxacin	5 µg	88(58.67%)	62(41.33%)
Nitrofurantoin	300 µg	114(76%)	36(24%)
High level gentamicin	120 µg	67(44.67%)	83(55.33%)
High level Streptomycin	300 µg	49(32.67%)	101(67.33%)
Vancomycin	30 µg	148(98.67%)	2(1.33%)
Teicoplanin	30 µg	149(99.33%)	1(0.67%)
Linezolid	30 µg	150(100%)	-

* Erythromycin is not reported in Urinary Isolates (CLSI 2015)

Among *E.faecalis*, Linezolid showed 100% susceptibility, followed by Teicoplanin (99.33%) and Vancomycin (98.67%), least susceptibility was with Pencillin G (24%), followed by High level Streptomycin (32.67%%), Erythromycin (39.68%).

TABLE – 13. ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *E.FAECIUM* BY KIRBY BAUER DISC DIFFUSION METHOD (n=78)

ANTIBIOTICS	Dosage Units	*E.faecium*	
		Sensitive	Resistant
Penicillin G	10U	14(17.95%)	64(82.05%)
Ampicillin	10 µg	32(41.03%)	46(58.97%)
Erythromycin	15 µg	12(28.57%)	42(71.43%)
Doxycycline	30 µg	45(57.69%)	33(42.31%)
Ciprofloxacin	5 µg	26(33.33%)	52(66.67%)
Levofloxacin	5 µg	42(53.85%)	36(46.15%)
Nitrofurantoin	300 µg	54(69.23%)	24(30.77%)
High level gentamicin	120 µg	31(39.74%)	47(60.26%)
High level Streptomycin	300 µg	26(33.33%)	52(66.67%)
Vancomycin	30 µg	74(94.87%)	4(5.13%)
Teicoplanin	30 µg	75(96.15%)	3(3.85%)
Linezolid	30 µg	78(100%)	-

∗Erythromycin is not reported in Urinary Isolates (CLSI 2015)

E.faecium showed higher suscpetibility to Linezolid (100%) followed by Teicoplanin (96.15%) & Vancomycin (94.87%) It showed lower susceptibility to Pencillin G (17.95%), followed by Erythromycin (28.57%), High level Streptomycin (33.33%), Ciprofloxacin (33.33%)

TABLE – 14 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF REST OF THE ENTEROCOCCAL SPECIES BY KIRBY BAUER DISC DIFFUSION METHOD:

ANTIBIOTICS	**Dosage Units**	***E.raffinosus* (n=6)**	***E.avium* (n=3)**	***E.durans* (n=2)**	***E.hirae* (n=1)**
Penicillin G	10U	2 (33.33%)	1(33.33%)	-	-
Ampicillin	10 µg	3 (50%)	1(33.33%)	1(50%)	1(100%)
Erythromycin	15 µg	2* (100%)	1*(100%)	1*(100%)	-
Doxycycline	30 µg	5 (83.33%)	2(66.67%)	2(100%)	1(100%)
Ciprofloxacin	5 µg	3 (50%)	2(66.67%)	1(50%)	-
Levofloxacin	5 µg	2 (33.33%)	2 (66.67%)	2(100%)	1(100%)
Nitrofurantoin	300 µg	5 (83.33%)	3 (100%)	2(100%)	1(100%)
High level Gentamicin	120 µg	3 (50%)	2 (66.67%)	2(100%)	1(100%)
High level Streptomycin	300 µg	2 (33.33%)	2 (66.67%)	1(50%)	1(100%)
Vancomycin	30 µg	6 (100%)	3 (100%)	2(100%)	1(100%)
Teicoplanin	30 µg	6 (100%)	3 (100%)	2(100%)	1(100%)
Linezolid	30 µg	6 (100%)	3 (100%)	2(100%)	1(100%)

* Erythromycin is not reported in Urinary Isolates - CLSI 2015.

Vancomycin, Teicoplanin & Linezolid showed highest susceptibility to all 4 species. Penicillin G is the least Susceptible to all 4 species

TABLE - 15 HIGH LEVEL AMINOGLYCOSIDE RESISTANCE IN ENTEROCOCCAL ISOLATES BY KIRBY BAUER DISC DIFFUSION METHOD

RESISTANCE PATTERN	***E.faecalis* (n=150)**		***E.faecium* (n=78)**	
	No. of Isolates	**%**	**No. of Isolates**	**%**
HLGR Alone	40	26.67%	7	8.97%
HLSR Alone	58	38.66%	12	15.38%
Both HLGR & HLSR (HLAR)	43	28.67%	40	51.28%

High Level Aminoglycoside Resistance is among 43 isolates (28.67%) in *E.faecalis* and among 40 isolates (51.28%) in *E.faecium*

TABLE-16 DISTRIBUTION OF VANCOMYCIN RESISTANT ENTEROCOCCI IN VARIOUS CLINICAL SPECIMENS (n=6)

SPECIMEN	**Total No. of Isolates**	**VANCOMYCIN RESISTANT *ENTEROCOCCI***		
		E.faecalis	***E.Faecium***	**Total**
Urine	131	1	2	3 (2.29%)
Pus	60	1	1	2(3.33%)
Blood	31	-	1	1(3.23%)
TOTAL	240	2	6	6(2.5%)

Among 6 VRE isolates, 3 were from Urine, 2 from Pus & 1 from Blood

TABLE -17 DISTRIBUTION OF VRE AMONG THE PATIENTS WITH RISK FACTORS / CO MORBID CONDITION (n=6)

ENTEROCOCCAL SPECIES	URINARY CATHETERISATION	BURNS	DIABETES	POST OPERATIVE INFECTION	SEPTICEMIA
Total Isolates	31	33	23	12	10
VRE	1	1	1	2	1

All 6 VRE isolates were associated with any one of these risk factors

TABLE18. PHENOTYPIC CLASSIFICATION OF VANCOMYCIN RESISTANT ENTEROCOCCI ISOLATES BASED ON MIC INTERPRETATION BY E-STRIP OF VANCOMYCIN AND TEICOPLANIN (n=6)

Van phenotype	***E.faecalis***	***E.faecium***	**Total**
van A Vancomycin MIC 32-64 µg/ml (R) & Teicoplanin MIC >16µg/ml (R)	1(50%)	3(75%)	4(66.67%)
van B Vancomycin MIC >8 µg/ml (IM/R) & Teicoplanin MIC 0.5-1 µg/ml (S)	1(50%)	1(25%)	2(33.33%)
Total VRE isolates	2(33.33%)	4(66.67%)	6(100%)

As per the above results, the vanA phenotype showing resistance to both vancomycin (64µg/ml) and teicoplanin (16 µg/ml) was observed in 4VRE isolates (66.67%). About 2 isolates belong to vanB (33.33%) (Phenotype with vancomycin MIC>8 µg/ml including the intermediate and resistant range and teicoplanin MIC in the susceptible range usually (0.5-1µg/ml) interpreted as per CLSI guidelines 2015.

CHART - VII

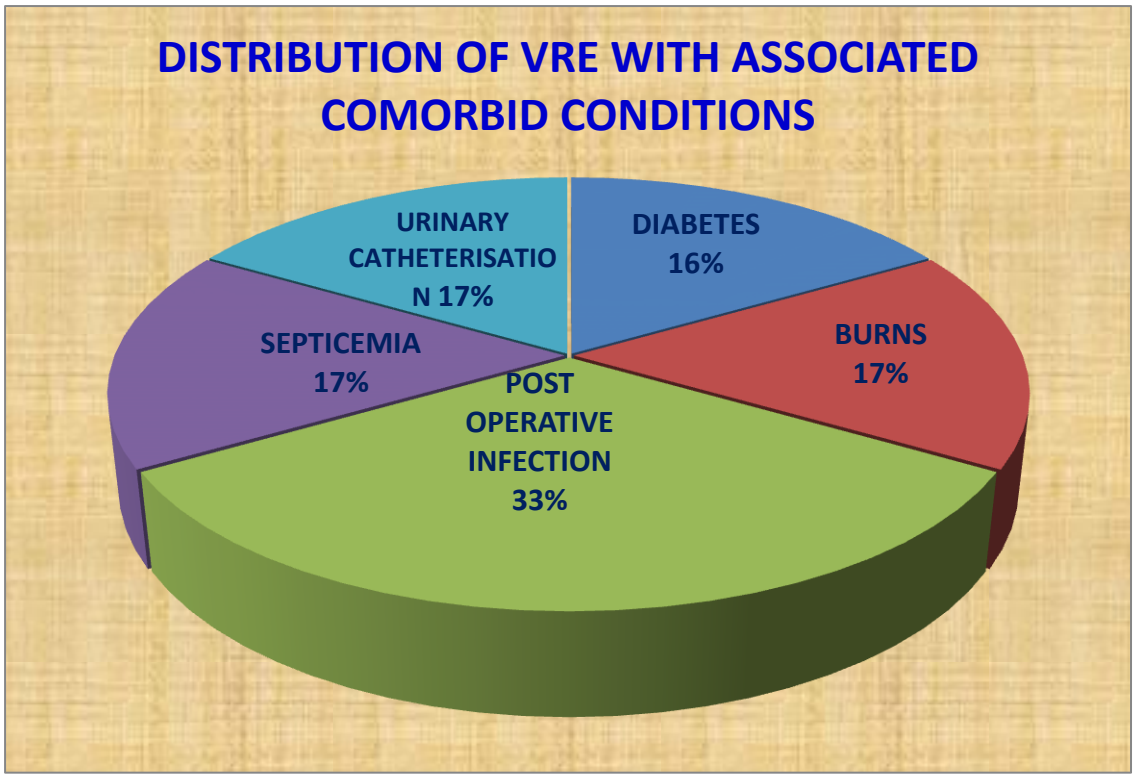


CHART - VIII

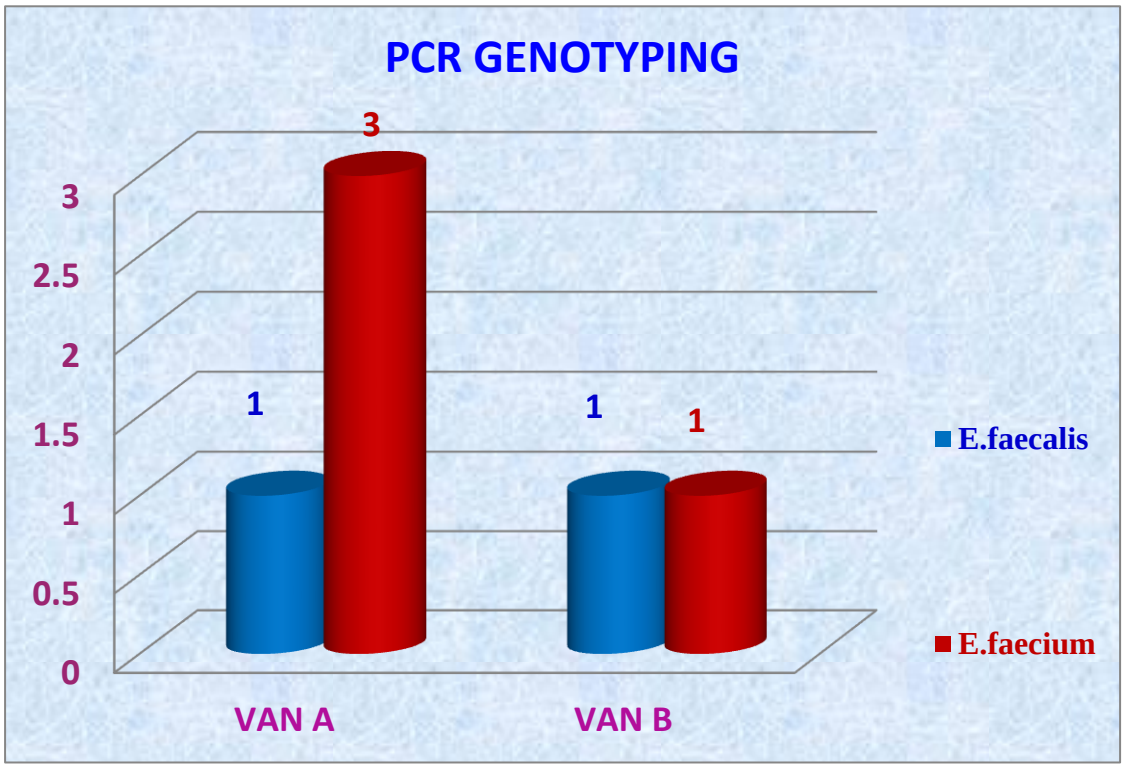


TABLE. 19 MIC VALUES FOR VANCOMYCIN FOR VANCOMYCIN RESISTANT ENTEROCOCCI SUSPECTED ISOLATES BY AGAR DILUTION METHOD (n=6)

VRE SUSPECTED ISOLATES (n=9)	VANCOMYCIN MIC VALUES IN (µg/ml)								TOTAL
	SENSITIVE	INTERMEDIATE		RESISTANT					
	<4 µg/ml	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml	512 µg/ml	
E.faecalis	-	-	-	1	1	-	-	-	3
E.faecium	-	-	-	1	1	1	-	1	6
TOTAL	-	-	-	2	2	1	-	1	9

MIC- Minimum inhibitory concentration

All the 6 VRE suspected isolates fall within the resistant range of 32-512µg/ml, interpreted as per CLSI guidelines 2015

TABLE - 20 THE CORRELATION OF MIC VALUES OF VANCOMYCIN WITH GENOTYPING FOR van A GENE (n=6)

VRE ISOLATES	NO. OF ISOLATES	MIC FOR VANCOMYCIN µg/ml		MIC FOR TEICOPLANIN µg/ml		PRESENCE OF van A GENE
		32-64 µg/ml	>64 µg/ml	<8 µg/ml	16-64 µg/ml	
E.faecalis	2	2	-	1	1	1
E.faecium	4	2	2	1	3	3
TOTAL	6	4	2	2	4	4

Among 6 VRE isolates 4 isolates showed the presence of van A gene. (1*E.faecalis* & 3 *E.faecium*)

TABLE-21 CHARACTERISTICS OF VANCOMYCIN RESISTANT ENTEROCOCCI ISOLATED IN OUR STUDY (n=6)

VRE ISOLATE	VRE SOURCE	ZONE DIAMETER (mm)		VANCO MYCIN SCREEN AGAR	MIC (µg/ml)		PCR GENOTYPE
		VANCOMYCIN	TEICPLANIN		VANCO MYCIN	TEICO PLANIN	
E.faecalis	Urine	R	S	R	32	0.5	van B
	Pus	R	R	R	64	32	van A
E.faecium	Urine	R	R	R	64	32	van A
	Urine	R	R	R	128	32	van A
	Pus	R	S	R	32	1	van B
	Blood	R	R	R	512	64	Van A

R- Resistant; S -Sensitive

Among 6 VRE isolates, 1 isolate of *E.faecalis* & 3 isolates of *E.faecium* showed vanA gene. 1 isolate of *E.faecalis* & 1 isolate of *E.faecium* showed van B gene.

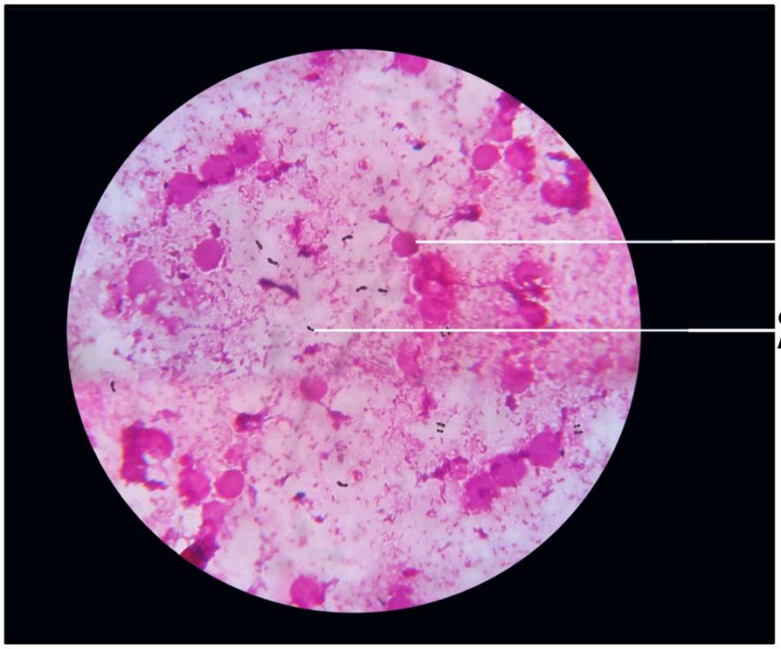
TABLE. 22- ANTIBIOTIC SUSCEPTIBILITY PATTERN OF VRE ISOLATES (n=6)

VRE ISOLATE	DISC DIFFUSION METHOD		
	LINEZOLID	QUINPRISTIN / DALFOPRISTIN	TIGECYCLINE
E.faecalis	2(100%)	Not Applicable	2(100%)
E.faecium	4(100%)	4(100%)	4(100%)
Total	6(100%)	4(100%)	6(100%)

Linezolid, Tigecycline showed 100% susceptibility to all 6 VRE isolates.

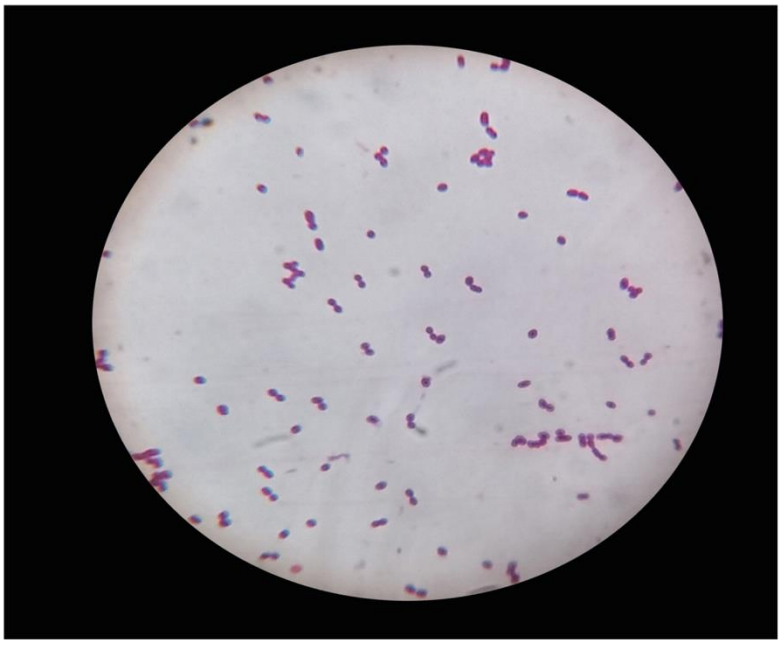
Quinipristin/Dalfopristin showed 100% susceptibility to all 4 VRE isolates of*Enterococcus faecium.*

DIRECT GRAM STAIN



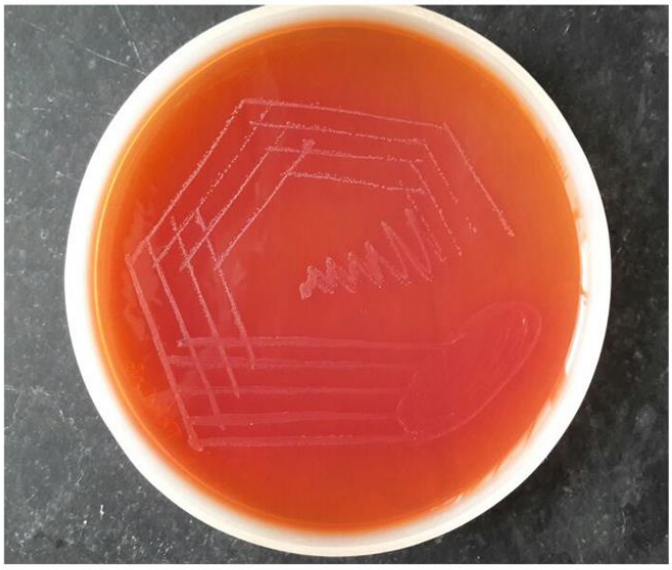
GRAM STAIN OF URINE SAMPLE SHOWING PLENTY OF PUS CELLS
AND GRAM POSITIVE COCCI IN PAIRS AND SHORT CHAINS

GRAM STAIN FROM CULTURE



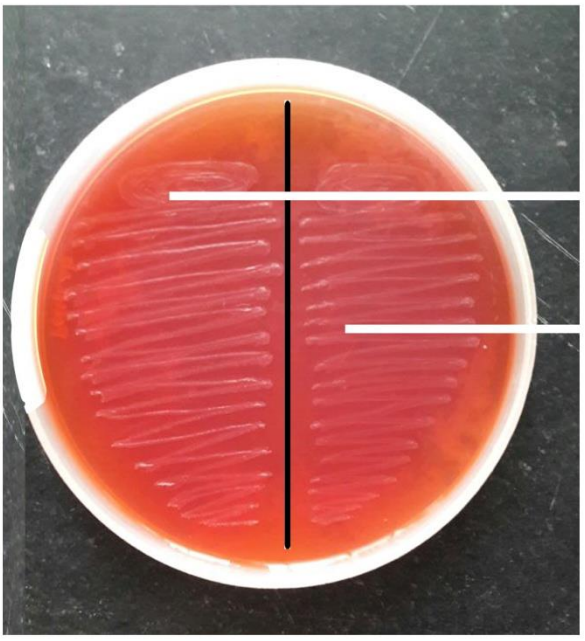
GRAM STAIN SHOWING GRAM POSITIVE COCCI
IN PAIRS AND SHORT CNAINS

MAC CONKEY AGAR PLATE SHOWING GROWTH OF ENTEROCOCCI

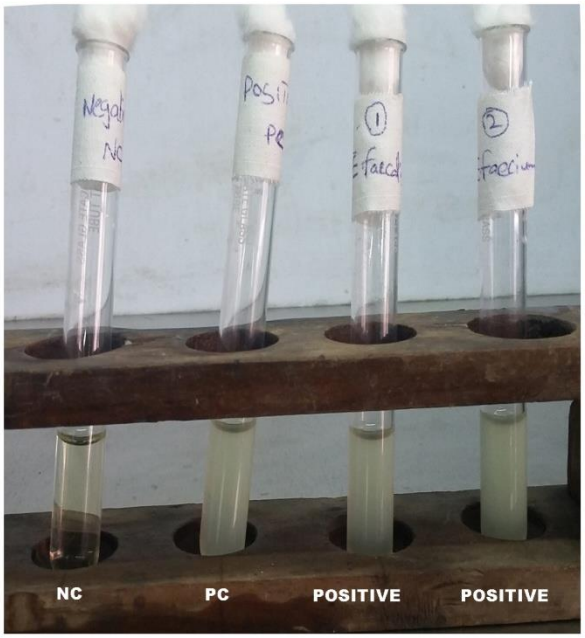


BILE ESCULIN HYDROLYSIS

HEAT TOLERANCE TEST

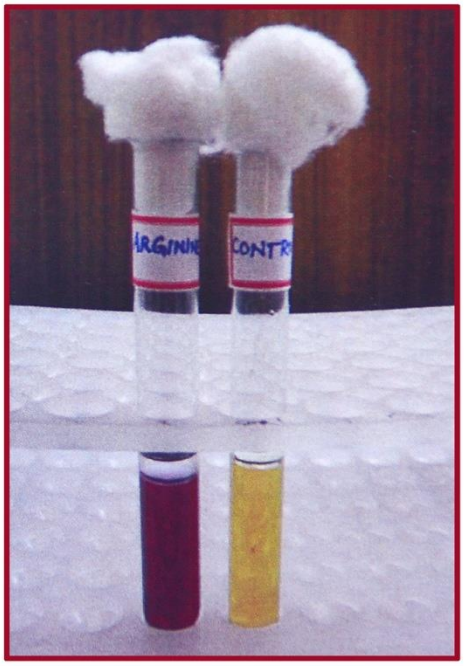


SALT TOLERANCE TEST



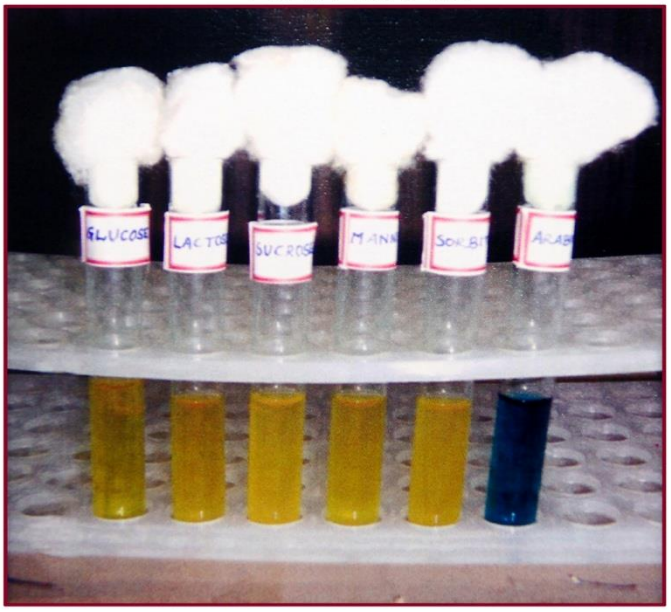
POTASSIUM TELLURITE REDUCTION

ARGININE HYDROLYSIS



ENTEROCOCCI SHOWING PURPLE COLOUR POSITIVE

CARBOHYDRATE FERMENTATION
E.FAECALIS



(Glucose+ ; Lactose+ ; Sucrose+ ; Mannose+ ; Sorbitol+ ; Arabinose -)

CARBOHYDRATE FERMENTATION
E.FAECIUM

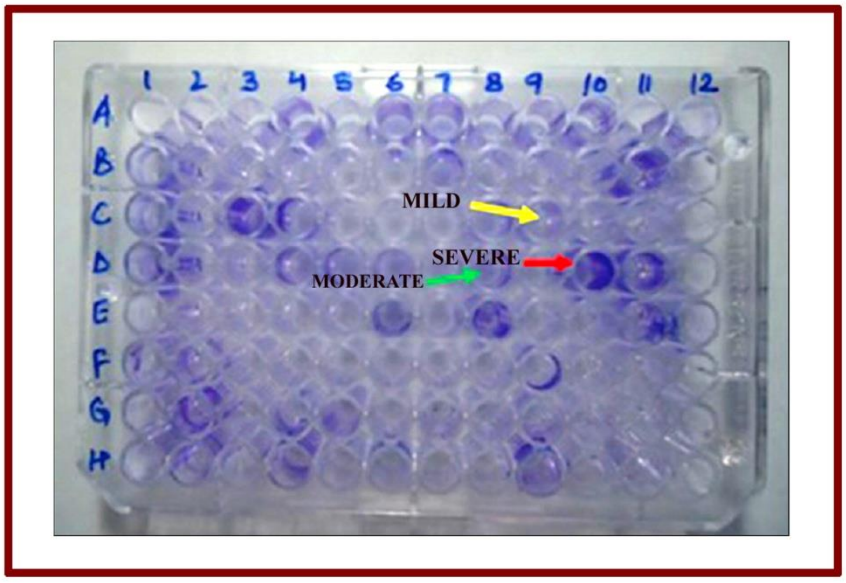
(Glucose+ ; Lactose+ ; Sucrose+ ; Mannose+ ; Sorbitol - ; Arabinose+)

VIRULENCE FACTORS PRODUCED BY ENTEROCOCCI

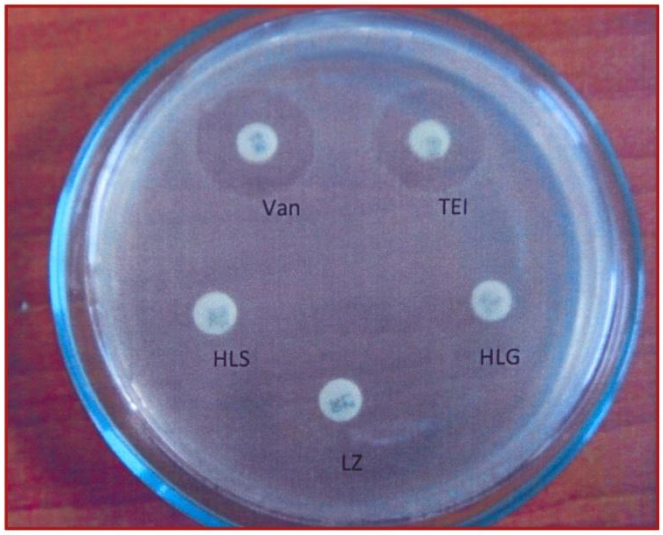
HEMOLYSIN PRODUCTION

GELATINASE PRODUCTION

BIOFILM PRODUCTION

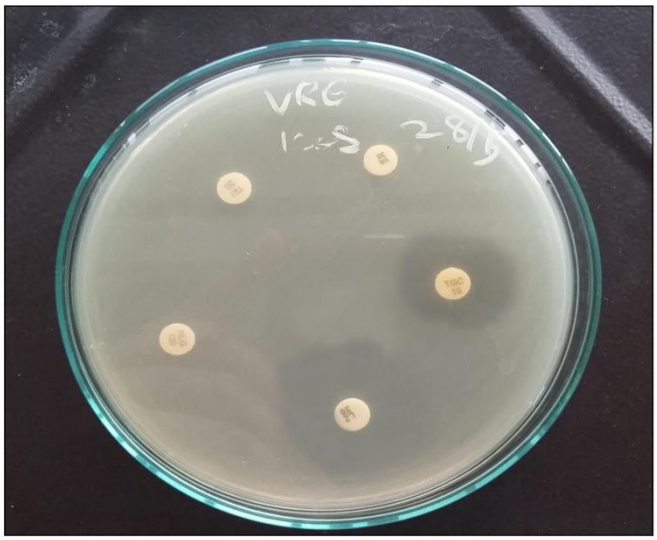


HIGH LEVEL AMINOGLYCOSIDE RESISTANCE ENTEROCOCCUS FAECIUM SHOWING RESISTANCE TO HIGH LEVEL GENTAMICIN & HIGH LEVEL STREPTOMYCIN



High Level Gentamicin-R; High Level Streptomycin-R;
Vancomycin-S; Teicoplanin-S; Linezolid-S

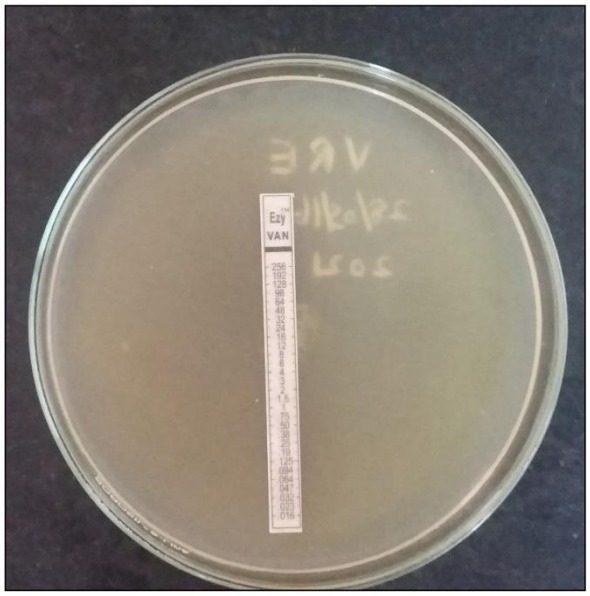
ANTIBIOTIC SUSCEPTIBILITY PATTERN FOR VANCOMYCIN RESISTANT ENTEROCOCCUS FAECALIS



Vancomycin-R; Teicoplanin-R; High Level Gentamicin-R;
Linezolid-S; Tigecycline-S

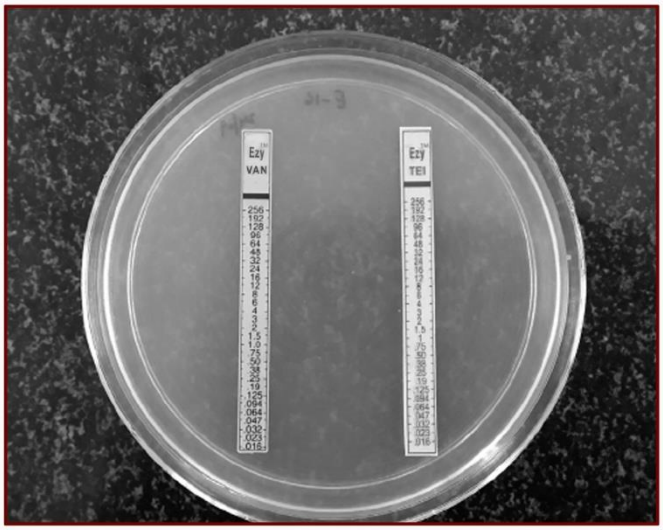
VANCOMYCIN SENSITIVE ENTEROCOCCI

VANCOMYCIN RESISTANT ENTEROCOCCI



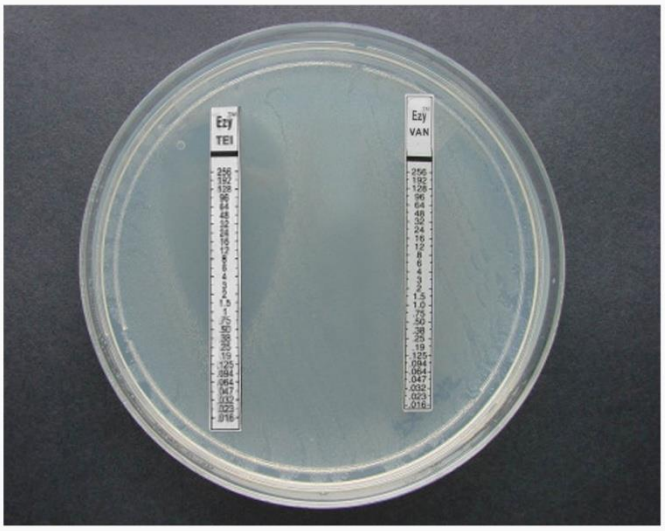
MINIMUM INHIBITORY CONCENTRATION DETERMINATION BY E-STRIP METHOD FOR VANCOMYCIN & TEICOPLANIN

van A PHENOTYPE



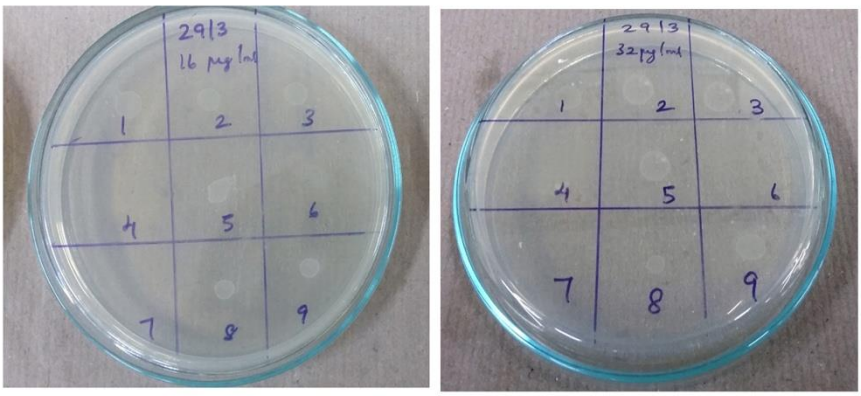
VANCOMYCIN-RESISTANT; TEICOPLANIN-RESISTANT

van B PHENOTYPE

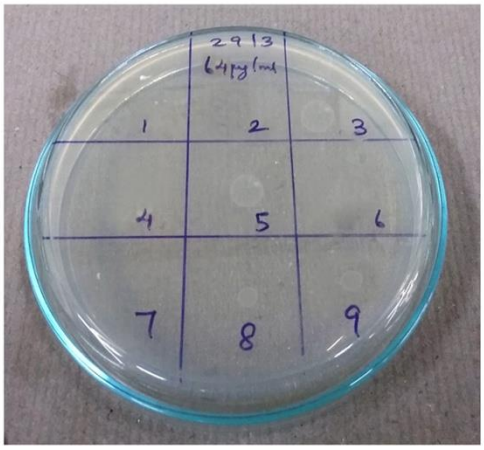


TEICOPLANIN-SENSITIVE; VANCOMYCIN-RESISTANT

MINIMUM INHIBITORY CONCENTRATION DETERMINATION BY AGAR DILUTION METHOD FOR VANCOMYCIN

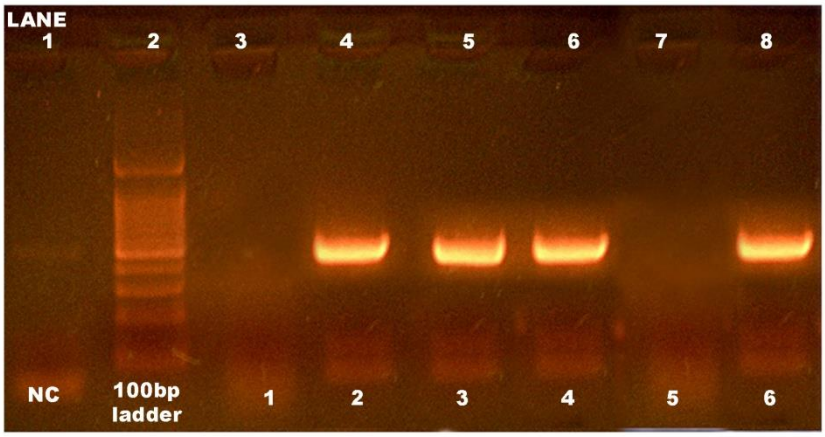


6 ENTEROCOCCAL ISOLATES SHOWING MIC 16 - 32 µg/ml



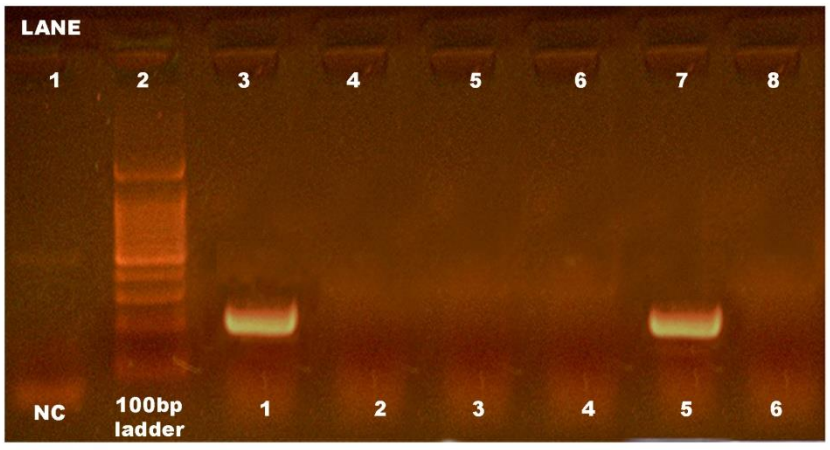
4 ENTEROCOCCAL ISOLATES SHOWING MIC ≥ 64 µg/ml

PCR - VAN A GENOTYPE



LANE 1 NEGATIVE CONTROL
LANE 2 100bp LADDER
LANE 3 - 8 SAMPLES WITH VAN A PRIMER
LANE 4,5,6,8 POSITIVE FOR VAN A GENE

PCR - VAN B GENOTYPE



LANE 1 NEGATIVE CONTROL
LANE 2 100bp LADDER
LANE 3 - 8 SAMPLES WITH VAN B PRIMER
LANE 3,7 POSITIVE FOR VAN B GENE

DISCUSSION

Enterococcus species have become important nosocomial pathogens worldwide and are associated with a high mortality[11]. *Enterococci* have assumed great clinical importance because of their increasing resistance to various antimicrobial agents[90.] Emergence of VRE is of concern due to the limited therapeutic options[11]. Hence it becomes essential to detect them at the earliest and to treat them with appropriate therapy based on the antimicrobial susceptibility pattern.

In our Study a total of about 240 *Enterococcal* isolates were isolated from Various clinical samples including urine, pus, blood, High Vaginal Swab.

As per table-1, *Enterococus* species were isolated from all age groups, it ranged from less than one year to 80 years, the youngest was 3 days old child and the oldest was of 80 years age. It was more isolated from 46-60 years 62(25.83%), followed by 16-30 years 61(25.42%), 31-45 years 56(23.33%). In a study done by Modi GB et al[84] where between age group of 61-75 Showing 30.40%, following which 46-60 years isolated 18.80%, 31-45 years isolated 15.20% which is in contrast to our study. But study done by D.Vijaya et al[49] in which *Enterococci* were isolated more among age 21-40 years followed by 40-60 yrs, which is also in contrast to our study.

As per our study in table -2, the total numbers of males were 129(53.75%) and females were 111(46.25%). The male to female ratio was 1.17:1. These findings are similar to studies done by Varun Goel et al[68], A.Tripathi et al[10], Modi

G.B et al[84] where *Enterococci* were more isolated from males and some studies show females were more infected than males like Maj Puneet Bhatt et al[90], D.Vijaya et al[49].

In this study it was shown in table-3 that we isolated *E.faecalis* as the predominant species 150(62.50%) followed by *E.faecium* 78(32.50%), *E.raffinosus* 6(2.50%), *E.avium* 3(1.25%), *E.durans* 2(0.83%) and *E.hirae* 1(0.42%). *E.faecalis* is the predominant species isolated in studies done by Jahnabi Barman et al[81] (81.72%), Maj Puneet Bhatt et al[90] (75%), Varun Goel et al[68] (53%) but in a study done by Jayavarthini Manavalan et al[6] they isolated *E.faecium* (52.38%) as the predominant isolate. M.sharifi-Rad et al[91]in their study isolated 7 *Enterococcal* species (*E.faecalis, E.faecium, E.raffinosus, E.avium, E.durans, E.hirae, E.mundtii*), also Desai et al[36] isolated 7 *Enterococcal* species (*E.faecalis, E.faecium, E.raffinosus, E.avium, E.hirae, E.casseliflavus, E.gallinarum*) which are similar to the species isolated in our study.

In our study as per table - 4, out of the 240 *Enterocooal* isolates the maximum isolates were from urine 131(54.58%) followed by pus 60 (25%), blood 31 (12.92%) and High Vaginal Swab 18 (7.50%). In the study taken by D.Vijaya et al[49] they isolated enterococci predominantly from urine(63%) , followed by pus(22%), blood(4%), high vaginal swab (3%) . In the study taken by Varsh Gupta et al[3] they isolated from urine(74.67%), pus(14.67%), blood(10.67%) and the results are similar to our study. In contrast were the studies done by Sanal.C.Fernandes et al[67] where they isolated from Urine (39.3%),followed by high

vaginal swab(35.3%), pus (15%), blood(2%) and Tripathi et al[10] in their study isolated from pus(50.81%) followed by urine(24.46%), blood (19.22%).

As per table -5 , a total of 31 (12.92%) *Enterococci* isolates were isolated from intensive care units (medicine, surgery), 180 (75%) were isolated from non ICU, 29(12.08%) were isolated from out patient Department. This is in contrast to studies done by Maj Puneet Bhatt et al[90] where they isolated from ICU (27%), Other wards (40%), OPD (33%) and Nonika Rajkumari et al[92] isolated *Enterococci* from ICU (63%), Other wards (37%). A study done by Modi G.B et al[84], where they isolated *Enterococci* from only 4.8% from ICU, 2.4% from Outpatient Department & 92.8%from other wards which is similar to our study that more *Enterococci* were isolated from other wards than ICU & OPD.

In Table -6, we discussed about the ward wise distribution of *Enterococci* isolates which was more from Surgical ward 79(32.92%), followed by Medical ward 37(15.42%), ICU(12.92%), Obstetrics & Gynecology (12.92%), Burns 22(9.17%). In contrast to our study findings observation were made by Modi G.B et al[84] , where they isolated *Enterococci* from Medical ward (38%), followed by Surgical ward(27.6%), Pediatric ward (14.40%), Gynecology ward (12.80%). They isolated only 4.8% from ICU & 2.4% from Outpatient Department.

In our study, in table-7 we observed that the risk factors were burns 33(13.75%), urinary Catheterization 31(12.92%), diabetes mellitus 23(9.58%), post operative wound Infection 12(5%), septicemia 31(12.92%). In a study done by A.Tripathi et al[10] they observed the following risk factors : urinary catheterization (46.5%), diabetes mellitus (31.8%), post operative wound infection (8.6%).

In table – 8, it shows correlation between Urinary catheterization and urinary tract infection. *Enterococcal* infection among catheterized patients were 28(21.37%) and non catheterized patients were 103(78.63%).It is in contrast to studies done by Sreeja et al[65] and P.J. Desai et al[36]. Sreeja et al[65] in their study observed 35% of UTI in patients with urinary catheterization and P.J. Desai et al[36] in their study noted only 8.92% of UTI from catheterized patients.

Table-9, shows the results of various biochemical tests used to differentiate *Enterococci* Isolates. Battery of 11 tests were done to identify the various species of *Enterococci*. Key tests involved in identification were acid production from Mannitol, sorbitol, arginine hydrolysis, pyruvate utilization. Along with key tests other differentiation tests also need to be done so as to not miss the correct identification of species. *E.faecalis* showed Lactose fermentation(96%), *E.faecium* showed Hippurate hydrolysis (41.02%), raffinose (35.89%),sorbitol (56.41%). *E.raffinosus* showed 80% to pyruvate utilization and *E.avium* showed 66.67% to Lactose fermentation. These results were in concordance with the study done by P.J Desai et al[36] where they used similar battery of tests for the speciation of *Enterococci*.

In our study as per table 10, study on virulence factors revealed production of hemolysin 18.33%, gelatinase 25.83% by *Enterococci*. This was similar to studies done by Jayavarthini M et al[6] which showed production of hemolysin 18.25%, gelatinase 19.84% and PM Giridhara et al[74] which showed production of

hemolysin 16 %, gelatinase 39%. Biofilm production in our study was 47.5%. It was in contrast to PM Giridhara et al [74] 32.5% and Jayavarthini M et al[6] 73.81%.

As per table -11, among co morbid conditions, virulence factors were more produced in post operative wound infection followed by burns wound infection , septicemia, diabetes, urinary tract infections. Hemolysin, Gelatinase, Biofilm were more produced in post operative wound infection. Biofilm was more produced in post operative wound infection (66.67%) followed by burns wound infection (60.61%) , septicemia(60%). Gelatinase was more produced in post operative wound infection (41.67%) followed bv diabetes (34.78%), burns wound infection (33.33%) , septicemia(30%). Hemolysin was more produced in post operative wound infection (33.33%) followed bv septicemia (30%), urinary tract infections (20.61%). In contrast, in a study done by P.M.Giridhara et al[74] they observed that virulence factors were more produced from urinary tract infection followed by Diabetes mellitus, post operative wound infection.

In this study according to Table-12, Among *E.faecalis*, Linezolid showed 100% susceptibility, followed by Teicoplanin (99.33%) and Vancomycin (98.67%), least susceptibility was with Pencillin G(24%), followed by High level Streptomycin (32.67%%), Erythromycin (39.68%). Similarly Linezolid showed 100% susceptibility in following studies Varun Goel et al[68], Jayavarthini M et al[6], Vijaya D et al[49], Sreeja et al[65]. In contrast resistance to Linezolid was shown by studies done by Nonika Rajkumari et al[92], Maj Puneet Bhatt et al[90]. In studies done by Sreeja et al[65], Jayavarthini M et al[6], Pencillin showed least susceptibility which

is similar to findings of our study. In contrast Ciprofloxacin showed least susceptibility in a study done by Vijaya D et al[49].

In this study according to Table-13, *E.faecium* showed higher suscpetibility to Linezolid (100%) followed by Teicoplanin (96.15%) & Vancomycin (94.87%) It showed lower susceptibility to Pencillin G (17.95%), followed by Erythromycin (28.57%), High level Streptomycin (33.33%), Ciprofloxacin (33.33%). This observation was similar to study done by Nonika Rajkumari et al[92]where they observed Pencillin followed by Erythromycin, Ciprofloxacin showing least susceptibility to E.faecium. Penicllin is the least susceptible in following studies done by Sreeja et al[65], Jayavarthini M et al[6], Vijaya D et al[49]. Linezolid is 100 % Susceptible in studies done by Jayavarthini M et al[6], Vijaya D et al[49], Sreeja et al[65], Nonika Rajkumari et al[92] which was similar to our study.

In this study according to Table-14, Vancomycin, Teicoplanin & Linezolid showed highest susceptibility to *E.raffinosus, Eavium, E.durans, E.hirae*. It is similar to the studies done by Jahnabi Barman et al[81], Agarwal.J, et al[83]. In contrast Vancomycin resistance were seen in studies for E.durans in Ghosh (Ray) Reena et al[88], *E.avium* in Sanal C.Fernandes et al[67]. Penicillin G is the least Susceptible to all 4 species is consistent with study done by Jahnabi Barman et al[81].

In this study table-15 showed high level aminoglycoside resistance among *Enterococcal* isolates by kirby bauer disc diffusion method. High Level Aminoglycoside Resistance was among 43(28.67%) in *E.faecalis* and among 40(51.28%) in *E.faecium*. High level Gentamicin resistance alone was shown among 40(26.67%) in *E.faecalis* and 7(8.97%) in *E.faecium*. Only High level

Streptomycin resistance alone was shown among 48(32%) in *E.faecalis* and 12(15.38%) in *E.faecium*. In our study among *E. faecalis* and *E. faecium* isolates resistant pattern was more to streptomycin than gentamicin, which is in contrast to study done by K.Suresh et al[80].

In table 16, totally 6 Vancomycin resistant *Enterococci* were isolated. Among 6 Vancomycin resistant *Enterococci*, 3 were from urine (1*E.faecalis* & 2*E.faecium*), 2 were from pus (1*E.faecalis* & 1*E.faecium*), 1 was from blood (1E.faecium). Thus a total of about 6 (2.5%) of both *E.faecium* and *E.faecalis* VRE isolates have been identified in our study. Agarwal.J, et al[83]have reported a VRE isolation rate of 4.65% in *E.faecalis* in their study. Ghoshal U et al[89] from Lucknow have reported a 1.4% VRE isolation (all were *E.faecium)* in their study which is lower than our study. Similarly Purva Mathur et al[86] isolated 1% VRE, Gupta et al[93] isolated 2% VRE. Similar to our study VRE isolates from urine, blood & pus were isolated in the study done by Kamarkar et al[66].

In our study table -17 we discussed about the distribution of Vancomycin resistant *Enterococci* among the patients with risk factors or co morbid condition. Among 6 Vancomycin resistant Enterococci, 2 were from Post operative infection followed by 1 each from Urinary tract infection, Burns wound infection, Septicemia, Diabetes mellitus. In contrast in a study done by A.Tripathi et al[10] where they isolated more VRE from patients with urinary catheterization followed by Diabetes mellitus.

Phenotypic classification of VRE isolates based on MIC interpretation by Epsilometer Strip of Vancomycin and Teicoplanin was discussed in Table 18. All

the 6 Vancomycin resistant isolates were tested for MIC of Teicoplanin and 2 isolates showed the MIC in the susceptible range (0.5-1 μg/ml) and 4 in resistant range (≥32μg/ml). Based on the MIC of vancomycin and teicoplanin, 4 VRE isolates (4/6) (66.67%) were of vanA phenotype showing resistance to both Vancomycin (32-256μg/ml) and Teicoplanin (32-64 μg/ml). The remaining 2 isolates belong to VanB phenotype (2/6) (33.67%) with Vancomycin MIC 32 μg/ml and Teicoplanin MIC in the susceptible range usually (0.5-1 μg/ml). The observations were similar to the one made by Ghosh (Ray) Reena et al[88] in their study. Ghoshal. U et al[89] where they observed Teicoplanin MIC range ≥32μg/ml in all their VRE isolates.

In our study as per table 19, Minimum Inhibitory Concentration was determined by agar dilution method for Vancomycin for VRE suspected isolates. All the 6 VRE suspected isolates fall within the resistant range of 32-512μg/ml. This observation was in similar to studies done by Ghosh (Ray) Reena et al[88], Agarwal.J et al[83].

In table 20, among 6 VRE isolates, High level resistance to both Vancomycin and Teicoplanin was shown by 3 *E.faecium* and 1 *E,faecalis* which belongs to van A phenotype. Thus there is 100% concordance of phenotypic classification by Vancomycin MIC and genotypic detection of the van A resistance type. Similar to our study, Suzanne et al[94 s]have reported 100% concordance of these two methods for the detection of van A VRE. Whereas Perlada.D et al[78] have reported 95% concordance in their study which is lower than that of our study. But

discrepancies between phenotypic & genotypic detection of van A, van B were shown in their study done by Ira prahraj et al[87], Rengaraj et al[9] .

In our study as per table 21, characteristics of vancomycin resistant *Enterococci* were discussed. Among 6 VRE isolates, vancomycin was resistant to all 6 isolates by both, disc diffusion method, Screen agar method. Four VRE isolates were resistant to Teicoplanin by disc diffusion method. We determined MIC by E-strip method which showed all 6 isolates were resistant to Vancomycin & 4 were resistant to Teicoplanin and we studied genotyping using PCR. It showed 4isolates were positive for van A genotype and 2 isolates were positive for van B genotype. It is similar to studies done by Ira prahraj et al[87], Purva Mathur et al[86] where they isolated van A genotype predominantly. van A is the most common genotype isolated in most of the studies including our study but in the studies done by Rengaraj et al[9], Ghosh(Ray) Reena et al[88] it was in contrast and van B gene was the most common gene isolated. Also in a study done by Kamarkar et al[66] they detected only van B gene from their 12 VRE isolates.

As per table 22, we observed the antibiotic susceptibility pattern of all VRE isolates with Linezolid, Quinpristin/Dalfopristin, Tigecycline using disc diffusion method. Interpretations were made by using CLSI 2015 & EUCAST 2015(for Tigecycline) guidelines. It showed that all the isolates were100% susceptibile to Linezolid and Tigecycline. Quinpristin/Dalfopristin was 100% sensitive to E.faecium producing VRE isolates. It was in concordance with Varsha Gupta et al[3] where they observed 100% susceptibility to Linezolid, Tigecycline, but reported resistance to Quinpristin/Dalfopristin in 2 of their isolates. In another study done by

Manoharan et al[95] where they showed similar findings as 100% susceptibilty to Tigecycline to all their VRE isolates. In a study done by Vidyalakshmi PR et al[96] they showed 100% susceptibility to Linezolid, Tigecycline. Linezolid resistance was reported in studies done by S.Rai et al[97], Maj Puneet Bhatt et al[90], and Yasliani et al[98]. Nonika Rajkumari et al[92] observed Linezolid and Quinpristin/Dalfopristin resistance in E.faecalis in their study.

Enterococci infection is really a concern for health care institution[99]. *Enterococci* may act as a reservoir for vancomycin resistance genes that could be transferred to more virulent bacteria, such as *Staphylococcus aureus* [11] Early detection of VRE and adherence to strict infection control measures will help in effective management and in preventing spread of Vancomycin resistant *Enterococci*[100]. Slowing the spread of vancomycin resistant *Enterococc*i (VRE) colonisation and infection, therefore, should remain a high priority for health care institutions.

SUMMARY

- *Enterococci* have emerged as important nosocomial pathogens with a capacity to cause a variety of infections and emergence of resistance to many of the antibiotics has made their management difficult.
- In this study, a total of 240 *Enterococcal* isolates were recovered from various clinical specimens. Majority of the isolates were from Urine 131(54.58%), followed by Pus 60(25%), Blood 31(12.92%), High vaginal Swab 18(7.50%)
- Among the isolates 25.83% were from 46-60 yrs age group followed by 25.42% in 15-30yrs, 23.33% in 30-45 yrs and 12.08% in 60-75 yrs. Isolates were more from male (53.75%) than female patients (46.25%).
- The isolates were speciated by conventional tests, of which *E.faecalis* is the predominant species 150(62.50%) isolated, followed by *E.faecium* 78(32.50%), *E.raffinosus* 6(2.50%), *E.avium* 3(1.25%), *E.durans* 2 (0.83%), *E.hirae* 1(0.42%).
- About 31 (12.92%) *Enterococcal* isolates were from intensive care units.
- *Enterococci* were isolated more from burns wound (13.75%) followed by Urinary catheterization (12.92%), Diabetes (9.58%), Post operative infection (5%), Septicemia (4.17%)
- Hemolysin 30(20%), Gelatinase 42(28%), Biofilm 78(52%) were produced by *E.faecalis* & Hemolysin 14(18%), Gelatinase 20(25.64%), Biofilm 33(42.30%) were produced by *E.faecium*. Virulence factors were produced

more in Post operative wound infection followed by Burns wound infection, Septicemia, Diabetes, Urinary tract infections.

- Antibiotic susceptibility was done by Kirby bauer disc diffusion method. *E.faecalis* isolates showed highest resistance to Penicillin 82.05% and least resistance to Teicoplanin 0.67% ,Vancomycin 1.33%. No resistance was seen with Linezolid and Tigecycline. *E.faecium* showed highest resistance to Penicillin 76% and least resistance to Teicoplanin 3.85% and Vancomycin 5.13%s. No resistance was seen with Linezolid, Quinipristin/Dalfopristin and Tigecycline.
- High Level Aminoglycoside Resistance was observed among 43 isolates (28.67%) in *E.faecalis* and among 40 isolates (51.28%) in *E.faecium*
- About 6 isolates (2.5%) were identified presumptively as vancomycin resistant by using Vancomycin screen agar containing 6µg/ml vancomycin.
- All the 6 isolates were tested for MIC for both Vancomycin & Teicoplanin by E-strip method, which showed all 6 isolates were resistant to Vancomycin (≥32µg/dl) and 4 isolates were resistant to Teicoplanin (≥16µg/dl). .
- MIC determination of Vancomycin using agar dilution method showed all those 6 isolates were resistant to Vancomycin (≥32µg/dl).
- About 4 VRE isolates showed MIC ≥64µg/ml for Vancomycin and ≥16µg/ml for teicoplanin and were identified as of van A Phenotype. The remaining 2 isolates showed Teicoplanin MIC in the range of 0.5-1µg/ml and were identified as van B Phenotype

- In PCR study, 4 vancomycin isolates showed van A gene and 2 isolates showed van B gene. van A was the commonest in both phenotypic & genotypic detection methods of Vancomycin resistance.
- We found in our study, 100% concordance between Phenotypic classification by Vancomycin MIC detection and molecular genotyping for the detection of van A type & van B type of VRE.
- All the 6 VRE isolates were sensitive to Linezolid and Tigecycline. All the 4 isolates of vancomycin resistant *E.faecium* were sensitive to Quinupristin and Dalfopristin.

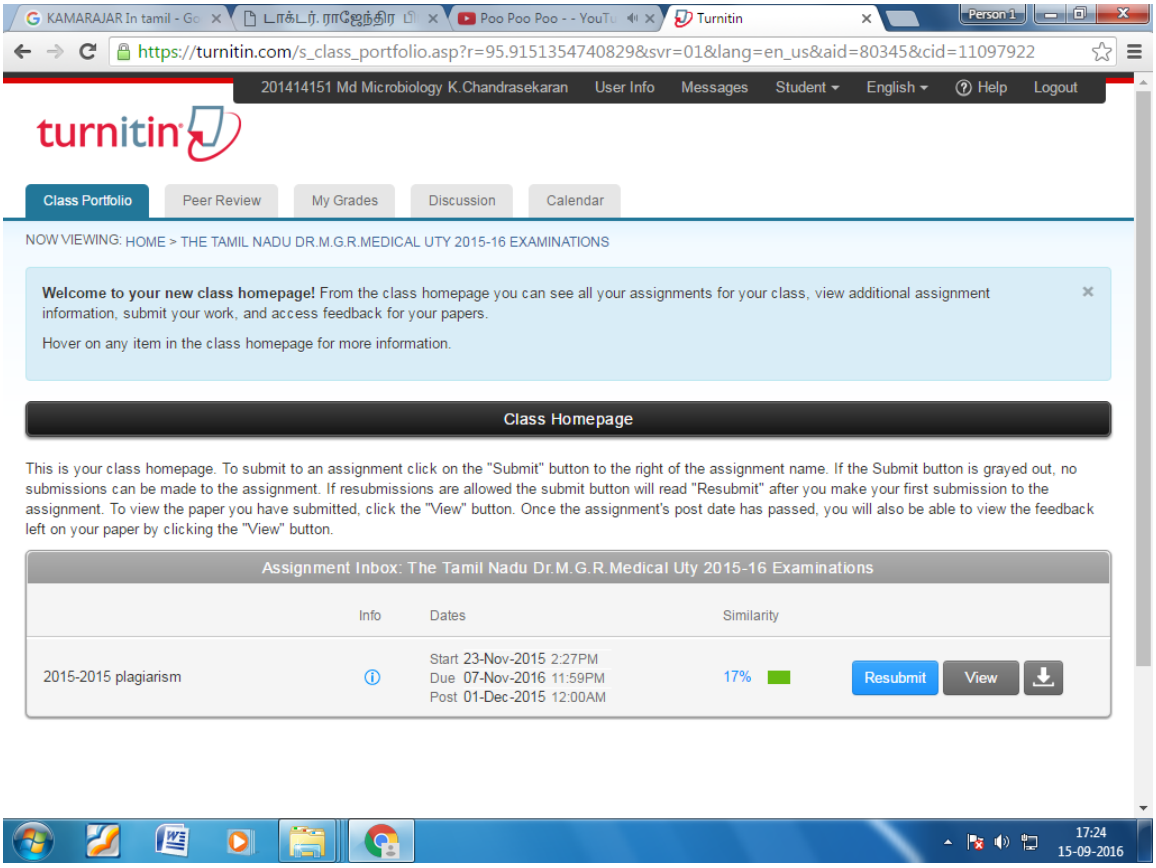
Enterococci have a incredible ability to survive in an environment of heavy antibiotics. Infact, it is their resistance to multiple antibiotics that makes them such feared opponents. Hence it warrants regular surveillance of antibiotic resistance of *Enterococci* and the implementation of an efficient infection control program in order to establish a rational antibiotic policy for the better management of *Enterococcal* infections.

CONCLUSION

Enterococci have become emerging nosocomial pathogens leading to complications, prolonged recovery or increased case fatality

- In our study we isolated a total of 240 *Enterococcal* isolates from various clinical samples among which *E.faecalis and E.faecium* were the predominant species isolated.
- Virulence factors like Hemolysin 44(18.33%), Gelatinase 62(25.83%), Biofilm 114(47.5%) were produced.
- They showed resistance to multiple antibiotics and all isolates were sensitive to Linezolid, Tigecycline, Quinipristin/ Dalfopristin
- 6 isolates of which 4 isolates were *E. faecium* & 2 isolates were *E.faecalis*, were identified as Vancomycin Resistant *Enterococci* with a prevalence rate of about 2.5% as per Vancomycin MIC and by PCR assay.
- In PCR study, van A gene was detected in 4 isolates and 2 isolates showed van B gene which was in concordance with both phenotypic & genotypic method of detection.

The present study showed emerging resistance of *Enterococci* which may lead to increase in morbidity & mortality that can be controlled by strict enforcement of antibiotic policy coupled with strict adherence to infection control measures to prevent further emergence and spread of antibiotic resistance.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INTRODUCTION
Enterococci are commensals of the gastrointestinal tracts of animals from simple invertebrates to humans[1]. They are known to be relatively avirulent in healthy individuals, but have become important opportunistic pathogens, especially in hospitalized patients[2].
Enterococci are recognized as opportunistic pathogens and are natural inhabitants of the oral cavity, gastrointestinal tract and the female genital tract in both humans and animals[3]. They have emerged as important nosocomial pathogens[3,4].
The most frequent infections caused by these organisms include urinary tract infections, intra abdominal and intra pelvic abscesses[3,5,6]. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis, and rarely, meningitis[5].
Although about 23 species of Enterococci have been identified, there are two main species, Enterococcus faecalis and Enterococcus faecium that are responsible for most human enterococcal infections[3,5,4]. Nevertheless, the incidence of other species of Enterococci is underestimated because of frequent misidentification[2].
Enterococcus was normally ignored by many clinicians in clinical samples as it is normal flora[7]. But now it stands individually in most of the recurrent infections and interferes with the healing of wound[7]. This organism has been reported as the second leading cause of
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INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10

Protocol ID No.07/12/2014 Dt. 20.01.2015

CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "Speciation, Characterisation and Antibiotic Susceptiblity pattern of Enterococci from Clinical isolates in a Tertiary care Hospital"- For Project Work-submitted by Dr.K.ChandraSekaran, MD (Micro Biology), PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

20/1/15

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

19/1/2015

GOVT. KILPAUK MEDICAL COLLEGE
20 JAN 2015
Kilpauk, Chennai-10.

PROFORMA

Name:

Age/Sex

Address:

Occupation:

Present Complaints:

Past History & Treatment:

Type of Clinical Sample:

Dept / Ward:

IP.No / OP No:

Lab No:

Date of Admission:

Diagnosis:

Microscopic Finding:

Gram Staining:

Culture Characteristics:

Blood Agar:

MacConkey Agar:

CLED Agar:

Biochemical Characteristics:

Sugar Fermentation Test:

Virulence Factors:

Hemolysin Production:

Gelatinase Production:

Biofilm Formation:

Antibiotic Susceptibility Pattern:

Signature of Investigator **Signature of Guide**

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு: **SPECIATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL**

ஆய்வு செய்யப்படும் இடம்: கீழ்பாக்கம் அரசு மருத்துவக்கல்லூரி மற்றும் மருத்துவமனை, சென்னை-10

பங்கு பெறுபவரின் பெயர்:
பங்கு பெறுபவரின் வயது: பங்குபெறுபவரின் எண் :

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கபட்டுள்ளது. நான் இவ்வாய்வில் தன்னிச்சையாக பங்கேற்கின்றேன். எந்த காரணத்தினாலோ, எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்றும் அறிந்துகொண்டேன்.

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவை இல்லை என அறிந்து கொள்கிறேன். இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ,முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன்.

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம்: சாட்சியாளரின் கையொப்பம்

இடம்: இடம்:

தேதி: தேதி :

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்:

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PATIENT CONSENT FORM

STUDY DETAIL: SPECIATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* FROM CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL

STUDY CENTER: KILPAUK MEDICAL COLLEGE AND HOSPITAL, CHENNAI.

PATIENT NAME: PATIENT AGE:

IDENTIFICATION NUMBER:

PATIENT TO TICK ( ) THESE BOXES

I conform that I have understood the purpose of procedure for the above study.

I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.

I understand that my participation in the study is voluntary and that I am free to withdraw at anytime without giving any reasons, without my legal rights being affected.

I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if withdraw from the study, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperative with the study team and immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.

I hereby give consent to participate in this study.

I hereby give permission to undergo complete clinical examination and diagnostic test.

Signature/Thumb impression: Place: Date:

Patient name and address:

Signature of the investigator: Place: Date:

Study investigator's name:

APPENDIX

PEPTONE WATER

Peptone	-	10g
Sodium chloride	-	5g
Distilled water	-	1 litre

Dissolve the ingredients in warm water, adjust the pH to 7.4 -7.5 and filter. Distribute as required and autoclave at 121 degree Celsius for 15 mins.

BLOOD AGAR

Sterile sheep blood	-	50 ml
Peptone	-	10 g
Beef extract	-	3g
Sodium chloride	-	5 g
Distilled water	-	1000 ml

Autoclave the nutrient agar base at 121º C for 15 minutes and blood with sterile precautions and distribute in Petri dishes.

MAC CONKEY AGAR

Peptic digest of animal tissue	-	17g
Proteose peptone	-	3g
Lactose	-	10g
Bile salts	-	1.5g
Sodium chloride	-	5g
Neutral red	-	0.03g

Agar	-	15g
Distilled water	-	1000 ml

Final pH at (25º C) 7.1±0.2.

Suspend 51.53 grams in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. Mix well and pour into Petri dish plates.

NUTRIENT AGAR

Peptic digest of animal tissue	-	5g
Beef extract	-	1.5g
Yeast extract	-	5g
Agar	-	15g
Distilled water	-	1000ml

Dissolve the contents in water and mix by heating. Autoclave at 121° C for 15 minutes. Adjust pH to 7.4 + 0.2. Pour 20-25 ml of 9 cm dia. Petri dishes to give 4 mm thickness

BILE ESCULIN AGAR:

Peptone	-	5 gm
Beef extract	-	3gm
Oxgall(bile)	-	40gm
Esculin	-	1gm
Ferric citrate	-	0.5gm
Agar	-	15gm
Distilled water	-	1 L

Final pH 7.0

Heat to dissolve the contents completely, sterilize at autoclave at 121ºC for 10 minutes, pour into slants/ Petri plates.

BRAIN HEART INFUSION BROTH:-

Calf brain infusion	-	200g
Beef heart infusion	-	250g
Proteose peptone	-	10g
Dextrose	-	2g
Sodium chloride	-	5g
Disodium phosphate	-	2.5g
Distilled water	-	1000ml

Add the contents and dissolve by heating. Adjust the pH to 7.4 + 0.2. Autoclave at 121° C for 15 minutes.

6.5% NaCl BROTH:

Nutrient broth	-	1L
NaCl	-	6.5gm

Dissolve the contents completely, autoclave at 121ºc for15 min and distribute in tubes.

MUELLER HINTON AGAR:

Beef infusion	-	300g
Casein acid hydrolysate	-	17.5g
Starch	-	1.5g
Agar	-	17g
Distilled water	-	1000ml

Final pH at 25º C 7.4.

Suspend 38 Gms in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 Minutes. Mix well and pour 20-25 ml of it into Petri dishes of 90 mm diameter to depth of 4 mm of medium.

MCFARLAND TURBIDITY STANDARD FOR INOCULUM PREPARATION

A Barium sulphate 0.5 McFarland standard was prepared as follows

1. A 0.5 ml of 0.048mol/L of Barium chloride was added to 99.5 ml of 0.18 mol/L of H2SO4 with constant stirring to maintain a suspension.

2. Correct density of the turbidity standard was verified by using a spectrophotometer. The absorbance of 625nm should be 0.08 to 0.10 for the 0.5 McFarland standards.

3. The Barium sulphate suspension was transferred in 4-6 ml to a screw capped tube of the same size as those used in growing or diluting the bacterial inoculum.

4. These tubes were tightly sealed and stored in the dark at room temperature.

5. The Barium sulphate turbidity standard was vigorously agitated before each use and inspected for a uniform turbid appearance

TABLE. 1. ZONE DIAMETER INTERPETIVE STANDARDS FOR *ENTEROCOCCUS* SPECIES. (CLSI GUIDELINES 2015)

Antimicrobial agent	Disk content	Zone diameter Interpretative criteria (Nearest whole mm)		
		Sensitive	Intermediate	Resistant
Penicillin G	10units	≥15	-	≤14
Ampicillin	10µg	≥17	-	≤16
Erythromycin	15µg	≥23	14-22	≤13
Doxycycline	30µg	≥16	13-15	≤12
Ciprofloxacin	5µg	≥21	16-20	≤15
Levofloxacin	5µg	≥17	14-16	≤13
Nitrofurantoin	300µg	≥17	15-16	≤14
High level Gentamicin	120µg	≥10	7-9	≤6
High level Streptomycin	300µg	≥10	7-9	≤6
Vancomycin	30µg	≥17	15-16	≤14
Teicoplanin	30µg	≥14	11-13	≤10
Linezolid	30µg	≥23	-	≤20
Quinpristin / Dalfopristin	15µg	≥19	16-18	≤15

TABLE-2. ZONE DIAMETER INTERPETIVE STANDARDS FOR *ENTEROCOCCUS* SPECIES. (EUCAST GUIDELINES 2015)

Antimicrobial agent	Disk content	Zone diameter Interpretative criteria (Nearest whole mm)		
		Sensitive	Intermediate	Resistant
Tigecycline	15µg	≥19	-	≤16

TABLE-3 MIC INTERPRETIVE STANDARDS FOR *ENTEROCOCCUS SPP.* (CLSI GUDELINES 2015)

Antimicrobial Agents	MIC Interpretive Criteria (µg/mL)		
	Sensitive	Intermediate	Resistant
Vancomycin	≤4	8-16	≥32
Teicoplanin	≤8	16	≥32

ABBREVIATION

ATCC	-	American Type Culture Collection
AFLP	-	Amplified-Fragment Length Polymorphisms
CAPD	-	Chronic Ambulatory Peritoneal Dialysis
CFU	-	Colony Forming Units.
CLED	-	Cystine Lactose Electrolyte Deficient
CLSI	-	Central Laboratory Standards Institute
CVC	-	Central Venous Catheters
ELISA	-	Enzyme Linked Immunosorbent Assay
E- TEST	-	Epsilometer Test
FDA	-	Food and Drug Administration (United States USFDA)
HLAR	-	High Level Aminoglycoside Resistance
HLG	-	High Level Gentamicin
HLS	-	High Level Streptomycin
ICU	-	Intensive Care Unit
OPD	-	Out Patient Department
MALDI –TOF	-	Matrix Assisted Laser Desorption/Ionisation – Time of Flight.
MDR	-	Multi Drug Resistance
MHA	-	Mueller Hinton Agar
MIC	-	Minimum Inhibition Concentration

MLST	-	Multi-locus Sequence Typing
MLVA	-	Multiple Locus Variable Number of Tandem Repeat Analysis
MRSA	-	Methicillin Resistant *Staphylococus aureus*
MRSE	-	Methicillin Resistant *Staphylococus epidermidis*
MSSA	-	Methicillin Sensitive *Staphylococus aureus*
MSCRAMM	-	Microbial Surface Components Recognizing Adhesive Matrix Molecules
NGS	-	Next Generation Sequencing
PCR	-	Polymerase Chain Reaction
PFGE	-	Pulsed-Field Gel Electrophoresis
PPE	-	Personal Protective Equipments
PYR	-	α-pyrrolidonyl β-naphthylamide
UTI	-	Urinary Tract Infection
VRE	-	Vancomycin Resistant *Enterococci*
VISA	-	Vancomycin Sensitive *Staphylococcus aureus*
VRSA	-	Vancomycin Resistant *Staphylococcus aureus*

KEY TO MASTER CHART

M	-	Male patient
F	-	Female patient
IP	-	Inpatient
OP	-	Outpatient
M (AGE)	-	Months
D (AGE)	-	Days
OG	-	Obstetrics & Gynecology
LBW	-	Labour Ward
ART CENTER	-	Anti Retroviral Therapy Center
ICU	-	Intensive Care Unit
NICU	-	Neonatal Intensive Care Unit
PICU	-	Pediatric Intensive Care Unit
BICU	-	Burns Intensive Care Unit
IMCU	-	Intensive Medical Care Unit
BPH	-	Benign Prostatic Hypertrophy
UTI	-	Urinary tract infection
POI	-	Post Operative Infection
CKD	-	Chronic Kidney Disease
PUO	-	Pyrexia of unknown origin

PO-TURP	-	Post Opeartive -Trans Uretheral Resection of Prostate
HVS	-	High Vaginal Swab
P	-	Produced
NP	-	Not Produced
S	-	Susceptible
R	-	Resistant
NA	-	Not Applicable
PEN – G	-	Penicillin G
AMP	-	Ampicillin
E	-	Erythromycin
CIP	-	Ciprofloxacin
LE	-	Levofloxacin
NIT	-	Nitrofurantoin
HLG	-	High Level Gentamicin
HLS	-	High Level Streptomycin
VA	-	Vancomycin
TEI	-	Teicoplanin
LZ	-	Linezolid
TIG	-	Tigecycline
RP	-	Pristinomycin

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Sl. No	Ip/Op No	IP/ OP	Lab No	Age	Sex	Ward/Dept	Diagnosis	Sample	Isolate	Virulence Factors			ANTIBIOTIC SUSCEPTIBILITY												
										Gelatinase	Hemolysin	Biofilm	PEN-G	AMP	DO	E	CIP	LE	NIT	HLG	HLS	VA	TEI	LZ	TIG	RP
1	1025	OP	33	24	F	Medical OP	UTI	Urine	E.faecalis	P	NP	P	S	R	S	R	S	S	S	S	S	S	S	S	S	NA
2	3527	IP	58	36	M	Surgery	POI	Urine(Catheterised)	E.faecium	P	P	P	S	S	S	S	S	S	S	R	R	S	S	S	S	S
3	1319464	OP	61	60	F	Medical OP	Diabetes	Urine	E.faecalis	NP	P	P	R	S	R	R	S	S	S	R	S	S	S	S	S	NA
4	1441338	OP	69	56	M	Medical OP	Fever	Urine	E.faecalis	NP	NP	NP	R	R	S	S	R	S	S	S	S	S	S	S	S	NA
5	1032/14	IP	75	60	M	Nephrology	UTI	Urine(Catheterised)	E.faecalis	P	NP	P	S	S	S	R	R	S	S	R	R	S	S	S	S	NA
6	843/14	IP	78	80	F	Nephrology	UTI	Urine	E.faecalis	NP	NP	NP	R	R	S	R	S	R	S	S	R	S	S	S	S	NA
7	21/15	IP	110	49	M	Nephrology	CKD	Urine	E.faecium	P	NP	NP	R	R	S	R	R	R	R	R	R	S	S	S	S	S
8	10061	OP	112	49	M	Medical OP	Diabetes	Urine	E.faecium	NP	NP	P	R	R	S	S	R	S	S	S	S	S	S	S	S	S
9	144/90	OP	161	22	F	Nephrology	UTI	Urine	E.faecalis	NP	NP	P	R	S	R	R	S	S	S	S	R	S	S	S	S	NA
10	4420	IP	174	55	F	Medicine	Fever	Urine	E.faecium	NP	NP	NP	R	S	S	S	S	S	S	S	S	S	S	S	S	S
11	144155	OP	198	19	M	Medical OP	PUO	Urine	E.faecalis	NP	NP	NP	R	S	R	R	R	R	S	S	S	S	S	S	S	NA
12	1438819	IP	230	41	M	Urology	POP-TURP	Urine (Catheterised)	E.faecium	NP	P	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S
13	1440060	IP	272	44	M	Nephrology	UTI	Urine	E.faecalis	NP	NP	P	R	R	S	R	R	R	S	R	R	S	S	S	S	NA
14	2371	OP	284	48	M	Urology	UTI/CKD	Urine	E.faecalis	P	P	P	S	S	S	R	R	S	S	S	S	S	S	S	S	NA
15	9783	IP	317	40	F	Medicine	PUO	Urine (Catheterised)	E.faecalis	NP	NP	NP	R	R	R	S	R	R	R	R	R	S	S	S	S	NA
16	86/15	OP	348	52	M	Urology	UTI	Urine	E.faecalis	NP	NP	NP	S	R	S	R	R	R	S	R	S	S	S	S	S	NA
17	8388	IP	365	25	M	Surgery	POI	Urine (Catheterised)	E.faecalis	P	P	P	R	R	R	S	R	S	R	R	R	S	R	S	S	NA
18	1673	IP	392	60	M	Nephrology	CKD	Urine	E.faecalis	NP	NP	P	R	R	S	R	R	R	R	R	R	S	R	S	S	NA
19	1968	OP	437	40	M	Urology	UTI	Urine	E.faecium	NP	NP	NP	R	R	S	R	R	R	S	R	S	S	S	S	S	S
20	1875	OP	447	60	F	Medicine	PUO	Urine (Catheterised)	E.faecalis	P	NP	NP	R	R	S	R	S	S	S	S	S	S	S	S	S	NA
21	2142	IP	454	51	F	Medicine	UTI	Urine	E.faecalis	NP	P	P	S	S	S	R	S	S	S	R	R	S	R	S	S	NA

22	2495	IP	458	55	M	Urology	UTI	Urine (Catheterised)	E.faecalis	NP	NP	NP	R	R	S	R	R	R	S	S	R	S	R	S	S	NA
23	113/15	IP	486	44	M	Urology	UTI	Urine (Catheterised)	E.faecium	NP	NP	P	R	R	R	R	R	R	R	R	R	S	R	S	S	S
24	575	IP	513	20	F	OG	PUO	Urine	E.faecalis	NP	NP	NP	S	S	R	R	R	S	R	R	R	S	R	S	S	NA
25	536	OP	537	30	M	Medical OP	PUO	Urine	E.faecalis	NP	P	NP	R	R	R	R	R	R	S	R	R	S	R	S	S	NA
26	2397	OP	618	55	F	Medical OP	Diabetes	Urine	E.faecium	NP	NP	NP	R	R	S	R	R	R	S	R	R	S	R	S	S	S
27	2896	OP	619	25	M	Medical OP	UTI	Urine	E.avium	NP	NP	NP	R	R	S	R	R	S	S	R	R	S	S	S	S	S
28	2715	OP	654	45	M	Medical OP	PUO	Urine	E.faecium	NP	NP	NP	R	R	S	R	R	S	S	S	R	S	R	S	S	S
29	2105	OP	662	38	F	Medical OP	Fever	Urine	E.faecalis	NP	NP	NP	R	R	S	R	R	R	S	R	R	S	R	S	S	NA
30	2622	IP	705	55	M	Medicine	UTI	Urine	E.durans	NP	NP	NP	R	R	S	R	R	S	S	S	S	S	S	S	S	S
31	2204	OP	710	60	M	Medical OP	PUO	Urine	E.faecium	NP	NP	NP	R	R	S	S	R	S	S	S	R	S	R	S	S	S
32	1284712	OP	752	22	F	Medical OP	PUO	Urine	E.faecalis	NP	NP	NP	R	R	S	S	R	R	S	S	R	S	R	S	S	NA
33	3224	IP	757	5 M	MC H	Pediatrics	Fever	Urine	E.faecalis	NP	NP	NP	R	R	R	R	R	R	S	S	R	S	R	S	S	NA
34	20048	IP	766	34	F	Surgery	UTI	Urine	E.raffinosus	NP	NP	NP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
35	20060	IP	798	35	M	Surgery	Fever	Urine (Catheterised)	E.faecalis	P	P	P	R	R	S	R	R	R	S	R	R	S	S	S	S	NA
36	14223	OP	847	47	M	Surgery	UTI	Urine	E.faecalis	P	NP	P	R	R	S	R	R	R	R	R	R	S	S	S	S	NA
37	3428	OP	853	62	M	Urology	PUO	Urine (Catheterised)	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
38	3204	IP	900	25	F	OG	Fever	Urine	E.faecium	NP	NP	NP	R	R	S	S	R	R	S	R	R	S	S	S	S	S
39	2197	IP	962	36	M	ART Center	Diarrhea	Urine	E.faecalis	NP	NP	NP	R	R	R	R	R	R	S	R	R	S	S	S	S	NA
40	3211	OP	965	62	M	Nephrology	UTI	Urine	E.faecalis	P	NP	P	R	R	R	R	R	R	S	S	R	S	S	S	S	NA
41	1109	OP	1070	40	M	Urology	UTI	Urine	E.faecalis	NP	P	NP	R	R	S	R	R	R	S	S	R	S	S	S	S	NA
42	208/15	IP	1082	63	M	Nephrology	PUO	Urine (Catheterised)	E.faecalis	NP	NP	NP	R	R	R	R	S	S	S	S	R	S	S	S	S	NA
43	4322	IP	1097	49	M	Urology	UTI	Urine (Catheterised)	E.faecalis	NP	NP	P	S	S	R	R	S	S	S	R	R	S	R	S	S	NA
44	4321	IP	1100	65	M	Urology	UTI	Urine	E.faecalis	P	NP	NP	R	R	R	R	R	R	R	R	S	S	S	S	S	NA
45	3320	IP	1124	6	MC H	Pediatrics	PUO	Urine	E.faecalis	NP	P	NP	R	R	R	R	R	R	R	R	R	S	R	S	S	NA
46	30051	IP	1154	55	F	Surgery	POI	Urine (Catheterised)	E.faecium	P	NP	P	R	R	R	R	R	S	S	S	S	S	S	S	S	S

47	117862	OP	1174	63	F	Medical OP	Fever	Urine	E.faecalis	NP	P	NP	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
48	4359	OP	1192	47	M	Medical OP	Fever	Urine	E.hirae	NP	NP	NP	R	R	R	R	R	S	S	R	R	S	S	S	S	S
49	327/15	IP	1196	68	F	Urology	UTI (Catheterised)	Urine	E.faecalis	NP	NP	P	R	R	R	R	R	R	R	R	R	R	S	S	S	NA
50	31860	OP	1212	11	MCH	Pediatrics	Fever	Urine	E.faecalis	NP	NP	NP	R	R	R	R	R	R	R	S	S	S	S	S	S	NA
51	4741	OP	1228	23	M	Urology	UTI	Urine (Catheterised)	E.faecalis	NP	NP	P	R	R	S	R	R	S	S	R	R	S	S	S	S	NA
52	236/15	OP	1231	48	M	Nephrology	PUO	Urine (Catheterised)	E.faecalis	NP	P	NP	R	R	S	S	R	R	S	R	R	S	S	S	S	NA
53	335	OP	1234	48	M	Urology	Diabetes	Urine (Catheterised)	E.faecalis	P	NP	P	S	S	S	S	S	S	S	S	S	S	S	S	S	NA
54	5502	IP	1257	30	F	OG/LBW	UTI	Urine	E.faecalis	NP	NP	NP	S	S	S	R	S	S	S	S	R	S	S	S	S	NA
55	32275	IP	1266	33	M	Surgery	UTI	Urine	E.faecalis	NP	P	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
56	5133	OP	1280	70	F	Medical OP	Fever	Urine	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	S	S	S	S	S	NA
57	5102	OP	1292	40	M	Urology	UTI	Urine (Catheterised)	E.faecium	NP	NP	NP	R	R	R	R	R	S	S	S	R	S	S	S	S	S
58	4381	IP	1322	36	F	Medicine	PUO	Urine	E.raffinosus	NP	NP	NP	R	S	S	S	S	S	S	R	R	S	S	S	S	S
59	112/15	OP	1336	47	M	Nephrology	UTI	Urine	E.faecalis	P	NP	P	S	S	S	S	S	S	S	S	S	S	S	S	S	NA
60	352	IP	1438	3	MCH	Pediatric ICU	PUO	Urine	E.faecium	NP	NP	NP	R	S	R	R	R	R	S	R	S	S	S	S	S	S
61	863	IP	1469	61	M	Urology	Post TURP	Urine (Catheterised)	E.faecalis	P	NP	P	R	R	R	R	R	R	S	S	R	S	S	S	S	NA
62	417/15	IP	1470	45	M	Urology	UTI	Urine	E.faecalis	NP	P	NP	R	R	R	R	S	S	S	R	R	S	S	S	S	NA
63	3214	IP	1514	70	F	OG	Post Hysterectomy	Urine	E.faecium	P	NP	P	R	R	R	R	R	S	S	R	R	S	S	S	S	S
64	424	OP	1560	59	M	Urology	Fever	Urine (Catheterised)	E.faecalis	NP	NP	NP	R	R	R	S	R	R	S	R	R	S	S	S	S	NA
65	42418	IP	1591	52	F	Surgery	UTI	Urine	E.faecalis	NP	NP	P	S	S	S	R	S	S	S	S	R	S	S	S	S	NA
66	431	OP	1604	24	F	Medicine	PUO	Urine	E.faecalis	NP	NP	NP	S	S	S	R	S	S	S	S	S	S	S	S	S	NA
67	5555	OP	1612	60	F	Urology	UTI	Urine	E.faecalis	NP	P	P	R	S	R	R	R	R	S	S	R	S	S	S	S	NA
68	769	OP	1620	34	F	Medicine	PUO	Urine	E.raffinosus	NP	NP	NP	R	R	S	R	R	R	S	R	R	S	S	S	S	S

69	463	OP	1643	21	M	Urology	UTI	Urine	E.faecium	NP	NP	NP	S	R	S	S	S	S	S	R	R	S	S	S	S
70	613	IP	1647	62	F	Medicine	UTI	Urine	E.faecalis	P	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
71	412	OP	1648	62	F	Urology	Renal Calculi	Urine	E.faecium	NP	NP	NP	R	R	R	R	R	S	S	R	S	S	S	S	S	S
72	798	OP	1655	49	M	Nephrology	Renal Calculi	Urine	E.faecalis	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
73	795	IP	1701	50	F	IMCU	Diabetic / UTI	Urine (Catheterised)	E.faecium	NP	P	P	R	R	S	R	R	S	S	R	R	S	S	S	S	S
74	5650	IP	1720	5	MCH	Pediatrics	PUO	Urine	E.faecalis	P	NP	NP	R	R	S	R	S	S	S	R	S	S	S	S	S	NA
75	45902	IP	1728	14	M	Surgery	UTI	Urine (Catheterised)	E.raffinosus	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S
76	5713	IP	1731	39	M	Nephrology	Renal Calculi	Urine	E.faecium	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S
77	1962	IP	1739	38	F	OG	UTI	Urine	E.faecium	NP	NP	NP	R	R	R	R	S	S	S	S	R	S	S	S	S	S
78	496/15	IP	1761	12	MCH	Urology	Renal Calculi	Urine	E.faecalis	NP	NP	P	S	S	R	R	S	S	S	S	R	S	R	S	S	NA
79	2142	OP	1772	31	M	Medical OP	UTI	Urine	E.faecium	NP	NP	NP	R	S	R	R	R	R	S	R	R	S	R	S	S	S
80	31854	OP	1773	3	MCH	Pediatrics	Fever For Evaluation	Urine	E.faecalis	NP	NP	NP	R	S	R	R	R	R	R	R	R	S	S	S	S	NA
81	6184	IP	1846	62	M	Medicine	PUO	Urine	E.faecium	NP	NP	P	R	S	R	R	R	S	S	R	R	S	S	S	S	S
82	5120	IP	1898	24	F	Urology	Renal Calculi	Urine	E.faecium	NP	NP	NP	R	R	R	R	R	R	R	R	R	S	S	S	S	S
83	3612	OP	1900	63	M	Medical OP	UTI	Urine	E.faecalis	NP	NP	P	R	S	R	R	R	R	S	R	R	S	S	S	S	NA
84	6413	OP	1901	27	F	Medical OP	Fever For Evaluation	Urine	E.faecalis	NP	NP	NP	R	S	S	R	R	R	S	R	R	S	S	S	S	NA
85	25608	IP	1913	50	F	Medical OP	UTI	Urine	E.faecalis	NP	NP	NP	R	R	R	S	S	S	S	S	S	S	S	S	S	NA
86	558/15	IP	1944	56	F	Urology	Renal Calculi	Urine	E.faecium	P	NP	NP	R	S	R	R	R	R	R	R	R	S	S	S	S	S
87	42354	IP	1952	64	M	Surgery	POI -UTI - Appendicitis	Urine (Catheterised)	E.faecium	P	P	P	R	R	R	R	R	R	R	R	R	R	R	S	S	S
88	1230	IP	1962	70	F	Surgery	Renal Calculi	Urine	E.faecalis	NP	NP	P	R	S	R	R	S	S	R	R	R	S	R	S	S	NA
89	6609	IP	1988	50	M	Medicine	Diabetes/ UTI	Urine	E.faecium	NP	NP	NP	R	S	S	R	R	R	R	R	R	S	R	S	S	S

90	31960	IP	1990	2	MCH	Pediatrics	Fever For Evaluation	Urine	E.faecalis	NP	NP	NP	R	R	R	R	S	S	S	R	R	S	R	S	S	NA
91	10400	OP	2020	77	F	Surgery	Stress Incontinence	Urine	E.faecalis	NP	NP	NP	R	S	S	R	R	S	S	S	S	S	S	S	S	NA
92	885	IP	2021	55	M	Medicine	Diabetes-UTI	Urine	E.faecium	NP	NP	P	R	R	R	R	R	R	R	R	R	R	R	S	S	S
93	45/15	IP	2027	79	M	Urology	PO-TURP	Urine (Catheterised)	E.faecalis	P	P	P	R	R	R	R	R	R	S	R	R	S	R	S	S	NA
94	56015	Op	2059	28	M	Medical OP	UTI	Urine	E.faecalis	NP	NP	P	R	S	S	R	R	S	S	R	R	S	R	S	S	NA
95	6986	OP	2061	24	f	Nephrology	UTI	Urine	E.faecalis	NP	NP	P	R	R	S	S	S	S	S	R	S	S	S	S	S	NA
96	65022	IP	2140	42	M	Urology	Renal Calculi	Urine	E.faecalis	NP	NP	NP	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
97	58895	IP	2150	60	F	OG	Stress Incontinence	Urine	E.faecalis	NP	NP	NP	R	S	R	R	S	S	S	S	R	S	S	S	S	NA
98	7153	IP	2155	58	F	Medicine	UTI	Urine	E.faecalis	NP	NP	P	R	R	S	R	R	S	R	R	R	S	S	S	S	NA
99	6471	IP	2160	30	M	Urology	Renal Calculi	Urine	E.faecalis	P	P	NP	R	R	S	S	S	S	S	R	R	S	S	S	S	NA
100	7377	IP	2183	73	M	Urology	Prostatitis	Urine	E.faecalis	P	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
101	7334	IP	2185	57	M	Urology	Stress Incontinence	Urine	E.faecium	NP	NP	NP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
102	7378	OP	2187	59	M	Medical OP	UTI	Urine	E.faecium	NP	NP	NP	R	R	R	R	R	R	R	R	S	S	S	S	S	S
103	7212	OP	2194	80	M	Urology	BPH Prostate	Urine	E.faecalis	NP	NP	NP	R	R	R	R	R	S	R	R	R	S	S	S	S	NA
104	7387	IP	2200	53	F	OG	Stress Incontinence	Urine	E.faecalis	NP	NP	P	R	R	R	S	R	S	S	R	R	S	S	S	S	NA
105	63950	IP	2262	65	M	Surgery	Renal Calculi	Urine	E.faecalis	NP	NP	NP	R	R	S	S	R	R	R	R	S	S	S	S	S	NA
106	657	IP	2270	80	M	Surgery	UTI	Urine (Catheterised)	E.faecalis	NP	NP	P	R	R	S	R	R	S	S	S	R	S	S	S	S	NA
107	2181	IP	2273	27	F	Urology	Renal Calculi	Urine	E.faecalis	NP	NP	NP	R	R	S	R	R	R	S	S	S	S	S	S	S	NA
108	3848	OP	2301	27	M	Medical OP	UTI	Urine	E.faecalis	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
109	52355	IP	2324	45	F	Surgery	UTI	Urine	E.faecalis	P	NP	P	R	S	S	S	S	S	S	R	R	S	S	S	S	NA
110	7918	IP	2340	54	F	Medicine	Diabetes	Urine	E.faecium	NP	NP	P	R	R	R	R	R	R	S	S	R	S	S	S	S	S

111	8015	IP	2349	59	M	Ortho	Traumatic Wound	Urine (Catheterised)	E.faecium	P	P	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S
112	7605	IP	2421	22	F	OG/LBW	Fever For Evaluation	Urine	E.faecalis	NP	NP	NP	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
113	8272	IP	2433	5	FCH	Pediatrics	Fever For Evaluation	Urine	E.faecalis	P	P	NP	R	R	R	R	R	R	R	S	S	S	S	S	S	NA
114	7089	IP	2503	30	F	OG/ LBW	Fever For Evaluation	Urine	E.faecalis	NP	NP	NP	R	R	S	R	R	R	R	S	R	S	S	S	S	NA
115	75344	IP	2574	35	F	Surgery	Dibetes / UTI	Urine (Catheterised)	E.faecalis	P	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
116	2958	OP	2623	35	M	Medical OP	Diabetes	Urine	E.avium	NP	NP	NP	R	R	R	R	S	S	S	S	S	S	S	S	S	S
117	80547	IP	2722	32	M	Urology	Renal Calculi	Urine	E.faecalis	NP	NP	NP	R	S	R	R	S	S	S	R	R	R	S	S	S	NA
118	78454	IP	2724	42	M	Urology	Renal Calculi	Urine	E.faecalis	NP	NP	P	R	S	R	S	S	S	S	R	R	R	S	S	S	NA
119	9590	OP	2776	10	MCH	Pediatrics	Fever For Evaluation	Urine	E.faecalis	NP	NP	NP	R	R	R	R	R	R	R	S	S	S	S	S	S	NA
120	9649	IP	2783	18	F	OG	UTI	Urine	E.faecalis	P	NP	P	S	R	S	R	S	S	S	S	R	S	S	S	S	NA
121	8432	IP	2852	75	M	IMCU	Diabetes/ UTI	Urine (Catheterised)	E.faecium	P	P	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S
122	196	IP	2870	30	F	Urology	Renal Calculi	Urine	E.faecium	NP	NP	NP	S	R	S	S	S	S	S	S	R	S	S	S	S	S
123	3124	OP	2935	65	F	Medical OP	Fever For Evaluation	Urine	E.faecium	NP	NP	NP	R	S	S	R	S	S	S	S	S	S	S	S	S	S
124	10192	OP	2943	57	M	Urology	BPH Prostate	Urine	E.faecalis	NP	NP	NP	R	S	S	R	R	S	S	R	S	S	S	S	S	NA
125	3384	IP	2969	50	F	OG	Stress Incontinence	Urine	E.faecalis	NP	NP	NP	R	S	R	R	R	R	S	R	S	S	S	S	S	NA
126	10588	OP	2986	35	F	Medical OP	Fever For Evaluation	Urine	E.faecalis	NP	NP	P	R	R	S	R	R	R	S	R	S	S	S	S	S	NA
127	833/15	IP	2994	28	M	Urology	Renal Calculi	Urine	E.faecium	NP	NP	P	R	S	R	R	R	R	S	R	R	S	S	S	S	S
128	844/15	IP	3014	46	M	Urology	BPH Prostate	Urine	E.faecalis	NP	NP	NP	S	S	S	S	S	S	S	S	R	S	S	S	S	NA

129	4120	OP	3076	70	M	Medical OP	Prostatitis	Urine	E.faecium	NP	NP	NP	R	R	S	R	R	R	R	R	S	S	S	S	S	S
130	860/15	IP	3084	59	M	Urology	BPH Prostate	Urine	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
131	8709	OP	1609	57	M	Surgery	Wound Infection	Pus	E.faecalis	P	P	NP	R	R	S	S	R	S	S	R	S	S	S	S	S	NA
132	9935	OP	1759	48	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	R	R	R	R	R	R	R	S	S	S	S	S	NA
133	12013	IP	2042	48	F	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	NP	P	R	R	S	S	S	S	S	R	R	S	S	S	S	NA
134	282/15	IP	2154	50	M	Surgery	POI	Pus	E.faecalis	P	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
135	3114	OP	2158	23	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	R	R	S	S	S	R	S	S	S	S	S	NA
136	12830	IP	2163	68	M	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	P	NP	R	R	S	S	R	S	S	R	R	S	S	S	S	NA
137	14119	IP	2268	32	F	BICU	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	S	S	S	R	S	S	S	S	R	S	S	S	S	S
138	12043	IP	2278	26	M	BICU	Burns Wound	Wound Swab	E.faecium	P	NP	NP	S	S	S	S	S	S	S	S	R	S	S	S	S	S
139	13772	IP	2304	37	M	BICU	Burns Wound	Wound Swab	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
140	13702	IP	2306	22	M	BICU	Burns Wound	Wound Swab	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
141	13422	IP	2335	36	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	R	R	R	R	R	R	S	S	S	S	S	NA
142	14721	IP	2463	23	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	S	S	S	S	S	S	S	S	S	S	S	NA
143	13902	IP	2469	60	M	Surgery	Peritonitis	Pus	E.faecalis	P	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
144	118674	IP	2540	70	M	Surgery	POI	Pus	E.faecium	P	NP	P	R	S	S	R	R	R	S	R	R	S	S	S	S	S
145	127331	IP	2681	50	M	Ortho	Wound Infection	Pus	E.faecium	NP	P	P	R	S	S	R	S	S	S	S	R	S	S	S	S	S
146	17388	OP	2768	21	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	S	S	S	S	S	S	S	S	S	S	S	NA
147	17427	OP	2771	26	F	OG	Vaginitis	HVS	E.faecalis	P	NP	NP	R	S	S	R	R	S	S	R	S	S	S	S	S	NA
148	14118	IP	2789	35	M	Surgery	Wound Infection	Pus	E.avium	P	NP	P	S	S	S	S	S	R	S	S	S	S	S	S	S	S
149	373	IP	1964	20	M	Surgery	POI-Appendicitis	Pus	E.faecalis	NP	P	P	R	R	R	R	R	R	R	R	R	R	R	S	S	NA
150	28131	IP	2248	64	F	Burns Ward	Wound Infection	Pus	E.faecalis	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA

151	18020	IP	2882	38	M	Surgery	Wound Infection	Pus	E.raffinosus	P	NP	P	S	S	S	R	R	S	S	S	S	S	S	S	S	S
152	17965	OP	2896	34	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	R	S	S	S	S	R	R	S	S	S	S	NA
153	18181	IP	2904	28	F	Burns Ward	Burns Wound	Wound Swab	E.faecalis	P	NP	P	S	S	S	S	S	S	S	R	R	S	S	S	S	NA
154	152290	IP	2935	17	M	Surgery	Wound Infection	Pus	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
155	18574	IP	2960	35	F	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	S	S	S	S	S	S	S	S	R	S	S	S	S	S
156	17170	IP	2968	25	M	BICU	Burns Wound	Wound Swab	E.faecalis	NP	NP	NP	S	S	S	S	S	S	S	R	R	S	S	S	S	NA
157	20212	OP	2985	18	M	Medical OP	Wound Infection	Pus	E.faecium	NP	NP	NP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
158	613	IP	2987	51	M	Ortho	Wound Infection	Pus	E.faecalis	NP	NP	P	R	S	S	R	R	S	S	R	R	S	S	S	S	NA
159	18114	OP	3014	21	F	OG	Vaginitis	HVS	E.faecium	NP	NP	NP	R	S	R	S	S	S	S	R	S	S	S	S	S	S
160	18516	IP	3032	30	F	OG	Wound Infection	Pus	E.faecium	P	NP	P	R	S	S	S	S	S	S	S	R	S	S	S	S	S
161	18673	OP	3065	27	F	OG	Vaginitis	HVS	E.faecium	NP	NP	NP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
162	19158	OP	3066	32	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	R	R	R	R	R	R	R	S	S	S	S	S	NA
163	20571	OP	3173	44	M	Burns Ward	Burns Wound	Wound Swab	E.faecalis	P	P	P	R	R	S	R	S	S	S	R	R	S	S	S	S	NA
164	20545	OP	3179	23	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	S	S	S	S	S	S	S	S	R	S	S	S	S	NA
165	20632	OP	3181	42	M	Medical OP	Wound Infection	Pus	E.faecium	NP	NP	NP	S	S	S	S	S	S	S	R	R	S	S	S	S	S
166	20784	OP	3185	74	M	Medical OP	Wound Infection	Pus	E.faecalis	NP	NP	P	S	S	S	S	S	S	S	S	S	S	S	S	S	NA
167	20138	OP	3203	21	F	OG	Vaginitis	HVS	E.faecalis	NP	P	NP	S	S	S	S	R	S	S	S	S	S	S	S	S	NA
168	20794	IP	3230	27	F	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
169	20873	IP	3233	75	M	Surgery	Diabetic Foot	Pus	E.faecium	NP	NP	P	R	S	S	R	S	S	S	S	S	S	S	S	S	S
170	17939	IP	3286	68	M	Surgery	Diabetic Foot	Pus	E.faecium	NP	P	NP	R	S	R	R	R	R	R	R	S	S	S	S	S	S
171	17449	IP	3314	60	F	Surgery	Wound Infection	Pus	E.faecalis	P	NP	P	R	R	S	R	R	R	R	R	R	S	S	S	S	NA

172	18003	IP	3315	48	M	Surgery	Diabetic Ulcer	Pus	E.faecium	NP	NP	P	R	S	S	R	R	R	S	R	R	S	S	S	S	S
173	119	IP	3335	58	M	Surgery	Wound Infection	Pus	E.faecium	P	NP	P	S	S	S	S	S	S	S	S	R	S	S	S	S	S
174		OP	3337	20	F	OG	Vaginitis	HVS	E.faecium	NP	NP	NP	R	S	S	R	R	R	S	R	R	S	S	S	S	S
175		OP	3362	22	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	R	S	R	S	S	S	S	S	S	S	S	S	NA
176	21276	IP	3372	55	M	Surgery	Diabetic Ulcer	Pus	E.faecalis	P	NP	P	S	S	S	R	S	S	S	S	S	S	S	S	S	NA
177	21667	IP	3380	40	F	Surgery	Wound Infection	Pus	E.faecium	NP	NP	P	R	S	R	R	R	S	S	R	R	S	S	S	S	S
178	21742	IP	3388	57	M	Burns Ward	Burns Wound	Wound Swab	E.durans	NP	NP	NP	R	S	S	R	S	S	S	S	R	S	S	S	S	S
179	21694	IP	3389	20	F	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	R	R	S	R	S	S	S	S	S	S	S	S	S	S
180	21559	IP	3390	1	MCH	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	NP	NP	R	R	S	R	S	S	S	S	S	S	S	S	S	NA
181	20232	iP	3391	32	M	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	NP	P	R	R	S	S	S	S	S	S	R	S	S	S	S	NA
182	21853	IP	3393	30	M	Burns Ward	Burns Wound	Wound Swab	E.faecium	P	NP	P	S	R	S	S	S	S	S	S	S	S	S	S	S	S
183	1073	IP	3394	55	M	Burns Ward	Burns Wound	Wound Swab	E.faecalis	P	NP	NP	R	R	S	R	S	S	S	S	S	S	S	S	S	NA
184	21432	IP	3401	32	F	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	P	NP	R	S	S	R	S	S	S	R	S	S	S	S	S	NA
185	21273	IP	3408	55	M	Surgery	Diabetic Ulcer	Pus	E.faecalis	P	NP	P	R	R	R	R	R	R	S	S	S	S	S	S	S	NA
186	21793	OP	3414	21	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	S	S	S	S	S	S	S	S	S	S	S	S	S	NA
187	21991	IP	3415	24	F	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	R	S	S	R	S	S	S	S	S	S	S	S	S	S
188	17326	IP	3416	30	F	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	NP	P	R	R	R	R	R	S	S	R	S	S	S	S	S	S
189	20820	IP	3418	37	F	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	P	NP	R	R	R	R	R	R	R	R	S	S	S	S	S	S

190	20016	IP	3419	36	F	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	P	P	R	R	S	R	S	S	S	S	R	S	S	S	S	NA
191	16487	IP	3421	53	F	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	NP	P	R	S	S	R	S	R	R	S	S	S	S	S	S	S
192	21969	IP	3422	4	FCH	Burns Ward	Burns Wound	Wound Swab	E.faecalis	NP	NP	NP	R	S	S	R	S	S	S	S	S	S	S	S	S	NA
193	21688	IP	3426	30	F	Surgery	Wound Infection	Pus	E.faecalis	P	NP	P	R	S	S	R	S	S	S	S	R	S	S	S	S	NA
194	21309	OP	3433	30	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	S	R	S	S	S	S	R	S	S	S	S	NA
195	22056	IP	3445	29	M	Burns Ward	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	R	S	S	R	R	S	S	S	S	S	S	S	S	S
196	21484	OP	3447	26	F	OG	Vaginitis	HVS	E.faecium	NP	NP	NP	R	R	S	R	R	S	S	S	S	S	S	S	S	S
197	21648	IP	3449	7	FCH	Pediatrics	Wound Infection	Pus	E.faecalis	NP	NP	P	S	S	S	R	S	S	S	S	S	S	S	S	S	NA
198	21884	IP	3450	3	FCH	Pediatrics	Wound Infection	Pus	E.faecalis	P	P	P	R	S	S	R	S	S	S	S	S	S	S	S	S	NA
199	22084	OP	3451	27	F	OG	Vaginitis	HVS	E.faecalis	NP	NP	NP	R	S	S	R	S	S	S	S	S	S	S	S	S	NA
200	21174	IP	3514	25	F	BICU	Burns Wound	Wound Swab	E.faecalis	NP	NP	NP	R	S	S	R	R	S	S	S	R	S	S	S	S	NA
201	22732	IP	3516	20	F	BICU	Burns Wound	Wound Swab	E.faecalis	NP	P	P	S	S	S	S	R	S	S	S	S	S	S	S	S	NA
202	22601	IP	3517	25	M	BICU	Burns Wound	Wound Swab	E.faecium	P	NP	NP	R	R	R	R	R	R	R	R	R	R	S	S	S	S
203	21154	IP	3518	40	F	BICU	Burns Wound	Wound Swab	E.faecalis	NP	NP	NP	R	S	S	R	R	S	S	S	S	S	S	S	S	NA
204	116862	OP	3520	56	M	Surgery	Diabetic Ulcer	Pus	E.faecalis	P	NP	P	R	R	R	S	R	R	R	R	R	S	S	S	S	NA
205	20825	IP	3524	36	M	Ortho	Wound Infection	Pus	E.faecium	NP	NP	NP	R	R	R	R	R	R	R	R	R	S	S	S	S	S
206	181673	OP	3527	60	M	Surgery	Diabetic Ulcer	Pus	E.faecium	NP	NP	P	S	S	S	S	S	S	S	S	S	S	S	S	S	S

207	484/15	IP	3532	45	M	Plastic Surgery	Wound Infection	Pus	E.faecium	P	P	NP	R	R	S	R	R	S	S	S	R	S	S	S	S	S
208	16238	IP	3548	33	M	BICU	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	R	R	S	R	R	R	R	R	R	S	S	S	S	S
209	16351	IP	3564	11	FCH	BICU	Burns Wound	Wound Swab	E.faecium	NP	NP	NP	R	R	R	R	R	R	R	R	R	S	S	S	S	S
210	3182	IP	240	49	F	IMCU	Septicemia	Blood	E.faecium	P	P	P	R	R	S	R	R	R	S	R	R	S	S	S	S	S
211	2615	IP	247	6D	FCH	NICU	Septicemia	Blood	E.faecalis	P	P	P	R	R	S	S	R	R	S	R	R	S	S	S	S	NA
212	2971	IP	280	48	F	Medicine	Fever For Evaluation	Blood	E.faecalis	NP	NP	NP	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
213	3779	IP	307	12 D	MC H	NICU	Septicemia	Blood	E.faecium	NP	NP	NP	R	R	S	S	R	S	S	S	R	S	S	S	S	S
214	4030	IP	327	6	MC H	Pediatrics	Fever For Evaluation	Blood	E.faecalis	NP	NP	P	R	R	S	S	S	S	S	R	S	S	S	S	S	NA
215	4458	IP	395	43	F	BICU	Burns Wound	Blood	E.faecium	NP	NP	P	R	R	S	S	S	S	S	R	R	S	S	S	S	S
216	4490	IP	397	36	F	Burns Ward	Burns Wound	Blood	E.faecalis	NP	NP	P	R	R	S	S	S	S	S	S	R	S	S	S	S	NA
217	250	IP	457	70	F	Nephrology	Diabetic/ UTI	Blood	E.faecium	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S
218	5525	IP	553	13 D	FCH	NICU	Septicemia	Blood	E.faecalis	NP	P	P	R	R	S	S	R	R	S	R	R	S	S	S	S	NA
219	6160	IP	555	3D	FCH	NICU	Fever For Evaluation	Blood	E.faecium	NP	NP	NP	R	R	S	S	R	S	S	R	R	S	S	S	S	S
220	5739	IP	559	7D	FCH	NICU	Septicemia	Blood	E.faecalis	P	NP	P	R	R	S	R	R	R	R	R	R	S	S	S	S	NA
221	6418	IP	575	22	F	OG	Fever For Evaluation	Blood	E.faecium	NP	NP	NP	R	R	R	R	R	R	R	R	R	S	S	S	S	S
222	6291	IP	583	7D	FCH	NICU	Fever For Evaluation	Blood	E.faecalis	NP	NP	NP	S	S	S	S	S	S	S	R	R	S	S	S	S	NA
223	7049	IP	633	60	M	Medicine	Renal Calculi	Blood	E.faecalis	NP	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA
224	7436	IP	634	24	M	Medicine	Diabetes/ UTI	Blood	E.faecalis	P	NP	P	R	R	R	R	R	R	R	R	R	S	S	S	S	NA

225	8145	IP	725	32	F	BICU	Wound Infection	Blood	E.raffinosus	NP	P	NP	R	R	S	R	S	S	S	S	R	S	S	S	S	S
226	10473	IP	932	32	F	BICU	Wound Infection	Blood	E.faecium	NP	NP	NP	R	R	S	S	R	R	S	R	R	S	S	S	S	S
227	11173	IP	958	38	F	Medicine	Fever For Evaluation	Blood	E.faecalis	NP	NP	NP	R	R	S	S	R	S	S	R	R	S	S	S	S	NA
228	11578	IP	961	10 D	FCH	NICU	Septicemia	Blood	E.faecalis	P	NP	P	R	R	S	S	R	R	S	R	R	S	S	S	S	NA
229	12258	IP	989	7D	FCH	NICU	Septicemia	Blood	E.faecium	P	P	P	R	S	R	R	S	S	S	R	R	S	S	S	S	S
230	10507	IP	1013	37	M	BICU	Wound Infection	Blood	E.faecalis	NP	NP	P	R	R	S	R	R	R	S	R	R	S	S	S	S	NA
231	13221	IP	1081	37	M	Medicine	UTI	Blood	E.faecalis	P	P	NP	R	R	S	R	S	S	S	R	R	S	S	S	S	NA
232	14749	IP	1166	64	M	Medicine	Fever For Evaluation	Blood	E.faecalis	NP	NP	NP	R	R	S	R	R	R	S	R	R	S	S	S	S	NA
233	17203	IP	1319	20	M	Medicine	Diabetes/ UTI	Blood	E.faecium	NP	NP	NP	S	R	S	S	R	S	S	R	R	S	S	S	S	S
234	16857	IP	1328	10	MCH	PICU	Septicemia	Blood	E.faecalis	NP	NP	P	R	R	S	R	R	R	S	R	R	S	S	S	S	NA
235	21078	IP	1593	63	F	Medicine	Diabetic Foot	Blood	E.faecium	P	NP	P	R	S	R	R	S	S	S	S	S	S	S	S	S	S
236	21493	IP	1654	23	F	Urology	POI	Blood	E.faecalis	P	NP	P	R	R	R	S	R	R	S	R	R	S	S	S	S	NA
237	22175	IP	1692	40	M	IMCU	Diabetes / UTI	Blood	E.faecium	NP	P	P	R	R	S	R	R	S	S	S	S	S	S	S	S	S
238	24265	IP	1907	32	M	IMCU	Septicemia	Blood	E.faecalis	P	P	P	R	R	R	R	R	R	R	R	S	S	S	S	S	NA
239	15267	IP	2048	54	M	Ortho	Septicemia	Blood	E.faecium	P	P	P	R	S	R	R	R	R	R	R	R	R	R	S	S	S
240	26702	IP	2115	35	M	BICU	Wound Infection	Blood	E.faecium	P	P	P	R	R	R	R	R	R	R	R	R	S	S	S	S	S